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Phosphorus Deficiency in the Rations of Cattle

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ILLUSTRATIONS

Dr. C. H. Eckles, Chief of the Division of Dairy Husbandry, died on February 13, 1933. His death is a serious loss to the Minnesota Agricultural Experiment Station and to the research project on phosphorus deficiency in cattle to which he has contributed so greatly. The general organization of this bulletin and those sections of it which came from his own pen represent Dr. Eckles' last major contribution to the science of dairying, in which field he has been a leading figure for many years.

L. S. Palmer
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PHOSPHORUS DEFICIENCY IN THE RATIONS OF CATTLE*

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INTRODUCTION

Since 1923 the Minnesota Agricultural Experiment Station has had under way a project to determine the cause of a severe deficiency that occurs in the rations of cattle in portions of Minnesota. In 1926 a report (1) (Bulletin 229, Minnesota Experiment Station) was published of results to that date indicating clearly that the trouble under investigation is the result of a phosphorus deficiency. In that bulletin is given an extended bibliography of the subject of mineral deficiencies with special reference to phosphorus.[1] Since Bulletin 229 appeared the authors have published several papers (2-12) in scientific journals giving results from this investigation.

It is the purpose of the present report to give a summary to date of the investigation regarding phosphorus deficiency as it occurs in Minnesota and adjoining states. As indicated in Bulletin 229, at the time the project was undertaken (December, 1923), the common view was that a mineral deficiency, if it occurred in the rations of cattle, would be the result of a shortage of calcium. By the end of the first year of our investigations (December, 1924), it became clear to us that we were dealing not with a calcium but with a phosphorus deficiency, and that calcium was present in ample amounts in the rations producing the abnormal conditions. After this fact had become clear, but before the publication of our report, Theiler and associates of South Africa issued their first report (13) of comprehensive investigations in the course of which they gathered ample evidence that the endemic condition they studied was likewise the result of a phosphorus deficiency.

During the six years since the publication of Bulletin 229, investigations have been continued on various phases of the problem. The

* The manuscript for this bulletin was submitted and approved for publication in August, 1932. A series of unfortunate circumstances is responsible for the delay in publication and accounts for the disparity between the date on the bulletin and the actual date of publication.

[1] A comprehensive review of the literature covering the field of phosphorus deficiency in relation to ruminants, prepared by Sir Arnold Theiler, is found in Nutrition Abstracts and Reviews, Vol. 1, No. 3, Feb. 1932, under the title "Aphosphorosis in Ruminants."

mass of data taken during this period confirms the conclusion given in Bulletin 229, that the cause of the trouble is a sub-normal intake of phosphorus. The source of the trouble is the low level of available phosphorus in the soil, as a result of which the forage grown does not provide enough of this element, especially when animals are maintained chiefly on roughage.

We reported in Bulletin 229 that typical deficiency conditions were produced by feeding forage grown in the areas where the trouble occurs and that badly affected animals had been brought back to normal by the use of phosphorus-bearing supplements.

The symptoms of phosphorus deficiency have been given in detail in other publications. Briefly, they are bone chewing; the gnawing of wood; a poor appetite, especially for roughage; stiffness in the joints; and poor physical condition, even when fed with reasonable liberality. Observations since the previous publication add only one important fact to the list of symptoms: In extreme cases of long standing, the early symptoms of chewing bone and gnawing wood disappear, and the animal, decidedly thin in flesh, becomes very inert, moving about little unless compelled to do so. An interesting and important fact is that our observations and tests indicate rather conclusively that bone chewing is a specific symptom of phosphorus deficiency. Animals fed on low-calcium rations, to the point even of showing tetany, do not exhibit any desire to chew bones.

Reports indicate this trouble is more or less prevalent in 30 counties of Minnesota. Since the publication of Bulletin 229, many additional reports have been received, and the area known to be affected is considerably increased. It should be made clear that symptoms of phosphorus deficiency do not appear among the cattle on every farm, even in the most affected area. The fact that certain herds do not develop the symptoms is probably to be attributed to more liberal feeding of concentrates, thus increasing the supply of phosphorus, or to the soil on that particular farm containing a more ample supply of phosphorus, which results in a higher phosphorus content of the forages grown. In other localities the reverse conditions prevail and the farm on which symptoms of deficiency develop is exceptional. Pronounced symptoms of phosphorus deficiency often prevail on a farm without the knowledge of the neighborhood or even without the situation being understood by the operator.

An occurrence of typical phosphorus deficiency in the southeastern quarter of Minnesota, heretofore considered free from this trouble, came to our attention recently. The farm in question is located on the typical prairie soil of this region. It has been under cultivation for about 60 years. So far as is known, the history is the same as that of other farms in the locality. Wheat was probably the chief crop for many years, followed by general farming, in which livestock and dairy cattle occupy the chief attention of the owner. No chemical fertilizer, so far as known, has been brought onto this land and little, if any, feed purchased. The soil, not especially high in phosphorus in the beginning, has become depleted of this element as a result of the system of farming followed, until now typical phosphorus deficiency has made its appearance among dairy cattle fed on its products. These observations suggest that phosphorus deficiency may be expected to appear in the future on many other farms where similar conditions have been maintained. As a result of the widespread publicity given the results of the investigation herein reported and active work by the staff of the Agricultural Extension Division, suitable methods of preventing the development of phosphorus-deficiency troubles have been adopted on a large percentage of the farms in Minnesota where the deficiency was formerly a serious obstacle to the development of a livestock industry.

The results of our experiments have been given wide publicity and as a result information regarding phosphorus-deficient areas has come to the authors by extensive correspondence and personal visits of interested persons as well as from publications. A single paragraph in a farm paper reporting these experiments brought 132 letters representing 30 states. It seems clear that in a considerable area in North Dakota, South Dakota, and Montana phosphorus-deficiency symptoms are common among cattle in certain years.

Brown,[2] in a report to a livestock association in Manitoba, states that out of 169 cattle owners in that Province answering a questionnaire 106, or 62 per cent, reported having experienced trouble with cattle the previous winter. The symptoms reported were such that it seemed certain to him that the trouble was phosphorus deficiency. Information received since the publication of Bulletin 229 also indicates that specific areas exist, the extent of which is not determined, where unmistakable phosphorus-deficiency symptoms occur in Wisconsin, Colorado, Utah, Nevada, New Mexico, and Florida. Hart (14) refers to bone chewing among cattle on the ranges in sections of California, and suspects a relation between the low intake of calcium and phosphorus by the animals in these regions and the small calf crop at times experienced.

[2] Copy to authors of report by Professor Brown of the University of Manitoba presented to the Manitoba Dairymen's Association.

MINERAL BALANCES IN PHOSPHORUS DEFICIENCY

During the first two years of the investigation the response of the animals to low-phosphorus rations and the addition of supplements was judged by physical symptoms and observation of the animals. Later mineral balance experiments were conducted to substantiate these observations. We employ the following procedure.

The urine and feces are collected together by making use of a metabolism stall constructed essentially after the plan devised at the Institute of Animal Nutrition, Pennsylvania State College. The cow stands on an elevated platform covered with heavy oiled canvas. Behind the cow is placed a galvanized iron container 17 inches wide and 15 inches deep with the length equal to the width of the stall. A removable shield protects both ends and the back edge against splashing. A flap of canvas hangs over the front edge making a water tight connection with the platform on which the animal stands. Distilled water is used to wash into the container any excreta dropped on the edge of the platform. During the collection period the animal is not kept under constant observation by attendants, but frequent visits are made to the stalls and the platform is washed when necessary. Little difficulty is experienced in obtaining a satisfactory collection. The excreta are weighed once each 24 hours and a sample is taken amounting to 5 or 10 per cent of the total. The daily samples are preserved either at room temperature with chloroform or in a refrigerated room without chloroform until the end of the 10-day period, when all are thoroly mixed, weighed, and a sample is taken for analysis. The final sample is preserved with a recorded amount of chloroform in a sealed Mason jar.

The feed is weighed out for the entire 10 days in advance of the beginning of the collection period to avoid fluctuations in the moisture content. The feed for each day is placed in an individual container. Refused feed is collected, dried in the air, and mixed into a single sample for analysis at the end of the collection period. The water drunk during the 10-day balance is also recorded.

The animals receive the experimental ration for a considerable time previous to the collection period. They are placed in the metabolism stalls from 3 to 5 days before the collection period begins in order to accustom them to the routine and the surroundings. During the first balances conducted, the animals were taken from the stalls and led for a short time in order to obtain exercise. Later it was decided this was not necessary or advisable, as experimental cows are accustomed to stand in the barn almost continuously, especially during the winter. We have found that if the cows are brushed thoroly with a stiff brush each day they seldom show any restlessness.

The methods of chemical analysis employed in connection with the balance trials are described in the appendix.

In this series, ten mineral balance trials are available in which five cows are represented. The roughage fed in all cases was low-phosphorus prairie hay grown on phosphorus-deficient soil. Attention was given to provide an amount of digestible protein and total digestible nutrients equal to, or slightly above, the requirements of the animal as indicated by the Morrison Feeding Standards (15). The results of two balance periods are available for each animal, one with a low or relatively low phosphorus intake, the other with essentially the same ration except a phosphorus-bearing supplement was added. In two cases (E 75 and E 93) the low-phosphorus ration was supplemented by calcium carbonate. Three of the cows used were non-lactating when the balances were taken and the other two were producing a fair amount of milk. The results are given in detail in Appendix tables 1 to 10 and a summary of conditions and results in the following statement.

Summary of Balance Trials

E 75, non-lactating; blood plasma, total Ca 12.92, inorganic P 1.75 mgm. per 100 cc. Low P hay, $CaCO_3$ supplement (Appendix table 1).

	Ca gm.	P gm.
Intake per day	65.54	6.58
Balance per day	−4.77	+0.05

E 75, non-lactating; blood plasma, total Ca 12.78, inorganic P 6.80 mgm. per 100 cc. Low P hay, $Ca_3(PO_4)_2$ supplement (Appendix table 2).

	Ca gm.	P gm.
Intake per day	61.53	27.59
Balance per day	+12.23	+2.88

E 93, non-lactating; blood plasma, total Ca 13.12, inorganic P 1.58 mgm. per 100 cc. Low P hay, $CaCO_3$ supplement (Appendix table 3).

	Ca gm.	P gm.
Intake per day	58.65	5.72
Balance per day	−9.31	−0.56

E 93, non-lactating; blood plasma, total Ca 12.13, inorganic P 4.94 mgm. per 100 cc. Low P hay, $Ca_3(PO_4)_2$ supplement (Appendix table 4).

	Ca gm.	P gm.
Intake per day	57.42	26.49
Balance per day	+14.74	+9.95

E 94, non-lactating; blood plasma, total Ca 13.68, inorganic P 0.77 mgm. per 100 cc. Low P hay, no supplement (Appendix table 5).

	Ca gm.	P gm.
Intake per day	25.55	6.59
Balance per day	+3.11	+1.19

E 94, non-lactating; blood plasma, total Ca 11.54, inorganic P 6.06 mgm. per 100 cc. Low P hay, $NaH_2PO_4 \cdot H_2O$ supplement (Appendix table 6).

	Ca gm.	P gm.
Intake per day	25.66	30.29
Balance per day	+15.37	+14.41

E 92, lactating (26.3 pounds milk daily); blood plasma, total Ca 10.92, inorganic P 5.08 mgm. per 100 cc. Low P hay, $NaH_2PO_4 \cdot H_2O$ supplement (Appendix table 7).

	Ca gm.	P gm.
Intake per day	26.56	43.97
Balance per day	−3.97	+5.39

E 92, lactating (22.8 pounds milk daily); blood plasma, total Ca 11.02, inorganic P 2.04 mgm. per 100 cc. Low P hay, no mineral supplement (Appendix table 8).

	Ca gm.	P gm.
Intake per day	25.11	18.69
Balance per day	−11.36	−4.03

E 74, lactating (19.6 pounds milk daily); blood plasma, total Ca 11.21, inorganic P 3.72 mgm. per 100 cc. Low P hay, $NaH_2PO_4 \cdot H_2O$ supplement (Appendix table 9).

	Ca gm.	P gm.
Intake per day	24.64	38.17
Balance per day	−7.09	+2.00

E 74, lactating (14.8 pounds milk daily); blood plasma, total Ca 11.12, inorganic P 3.82 mgm. per 100 cc. Low P hay, no supplement (Appendix table 10).

	Ca gm.	P gm.
Intake per day	27.77	17.71
Balance per day	−8.18	−0.75

It will be noted from these results that E 75, E 93, and E 94, non-lactating cows receiving the low-phosphorus ration, were close to a phosphorus balance, two showing a slight negative and one a positive balance of 1.19 grams per day. These results confirmed our observations that mature non-lactating cows may obtain enough phosphorus for maintenance from the low-phosphorus intake as supplied by a ration composed chiefly of prairie hay grown upon phosphorus-deficient soil. The grain ration was limited to oats to conform to the most common feeding practice in the low-phosphorus areas in Minnesota.

The rather striking positive phosphorus balances obtained when the same animals received phosphorus-bearing supplements corroborated our observations that the symptoms of phosphorus deficiency disappear in every case within a short time when a phosphorus supplement is added to the ration.

Two balance trials were made with lactating cows on a ration of low-phosphorus hay and oats. Of these, E 92, producing 22.8 pounds of milk daily, was in a negative balance of 4 grams daily. Cow E 74, producing but 14.8 pounds of milk, showed only a small negative balance. This small negative result is probably explained by the relatively large amount of oats received, 11 pounds per day. In order to meet the feeding standard requirement it was necessary to supply this amount of grain, which is decidedly more liberal feeding than commonly practiced in the phosphorus-deficient area in Minnesota. This liberal grain intake supplied a fair amount of phosphorus in the ration, regardless of the low-phosphorus content of the hay. In both cases a decidedly positive balance was obtained with the same lactating cows when the phosphorus supplement was given.

EXPERIMENTAL PRODUCTION OF PHOSPHORUS DEFICIENCY

Our experimental work was begun, as pointed out in a previous publication, by bringing cattle from affected farms to the experiment station. In the first experiment, begun before the nature of the deficiency was understood, cows were purchased from a farm where experience had demonstrated that the typical symptoms might be expected to develop each winter. The cows were brought to the experiment station in December. No abnormal symptoms could be observed at this time, altho these animals had been affected the previous winter. Freedom from symptoms at this season is typical of the observations and experience of farmers in the region where the trouble occurs. Feed was brought from the same farm. Two of the group received the typical ration fed on the farm from which they came, prairie hay and oats without supplements. The following figures show the average daily feed intake and milk production during the first 180 days:

Cow	Weight	Daily milk flow	Prairie hay daily	Oats daily	Ca daily	P daily
	lb.	lb.	lb.	lb.	gm.	gm.
E 32........................	914	14.0	23.7	6.1	62.25	19.66
E 33........................	665	7.2	17.1	3.7	44.01	13.00

The prairie hay contained 0.572 per cent calcium and 0.087 per cent phosphorus; the oats 0.136 per cent calcium and 0.349 per cent phosphorus.[3] With this ration, one cow first showed definite signs of a desire to chew bones at 186 days, the other at 194 days. Undoubtedly these animals had a limited storage of phosphorus because of having been subject all their lives to a low level of intake. According to their former owner, both had been persistent bone chewers the previous winter. In addition to the typical symptom of bone chewing,

[3] All chemical analyses reported in this bulletin are on the air-dry basis unless otherwise stated.

they gradually assumed an unthrifty appearance and lost some weight, even tho the intake of nutrients was above the amount specified by common feeding standards as sufficient to maintain their weight and their milk production. These symptoms are also typical of animals observed in the phosphorus-deficient region. However, the conditions developed were mild compared with those reported and observed on numerous farms. It was not until later that the conditions involved were thoroly understood and typical cases of severe phosphorus deficiency were developed in animals raised on the University farm and fed regular market feedstuffs.

The greatest demand for calcium and phosphorus is during growth and lactation. A decidedly moderate intake is sufficient to maintain a non-lactating mature animal. The conditions necessary to develop extreme phosphorus deficiency are to limit the ration to feedstuffs sufficiently low in phosphorus, and to continue this feeding a sufficient length of time. The response is much quicker with a growing animal or a lactating cow.

The basis of the rations used that resulted in extreme phosphorus deficiency was prairie hay grown on phosphorus-deficient soils. Analyses of a large number of samples are given in Appendix tables 15 and 16. Fifty-one samples of hay of this class, gathered in the phosphorus-poor soil area, showed an average phosphorus content of 0.106 per cent. The hay of this class used in our experimental work averaged 0.071 per cent of phosphorus for 21 samples, the detailed analyses of which are given in Appendix table 16. A lactating cow receiving roughage of this type with a limited intake of common grains or corn will inevitably begin to show symptoms of phosphorus deficiency after a few months in lactation—a lowered content of inorganic P in the blood plasma will generally appear within three months. Before the end of a normal lactation period, pica, one of the typical symptoms of phosphorus deficiency, should appear.

Other varieties of hay, especially timothy, grown on a low-phosphorus soil may be expected to give the same results. Dried beet pulp, which may be used to supply the necessary roughage, has a phosphorus content about the same as prairie hay grown on phosphorus-deficient soils. Eight lots of the dried pulp used in the experiments reported had an average phosphorus content of 0.082 per cent. Black strap molasses (a by-product from the manufacture of cane sugar) also carries approximately the same phosphorus percentage. Corn gluten meal has been found to serve as a useful source of protein in preparing a low-phosphorus ration. A range of over 100 per cent has been found in the phosphorus content of different brands of gluten meal at present on the market due, presumably, to the methods of manufacture.

The amount of phosphorus stored in the animal is also a factor of great importance in connection with the time required to initiate phosphorus-deficiency symptoms. The first evidence regarding this point was obtained from a farmer in the affected area who stated that he had observed that cows brought to his farm from farms on another type of soil did not show the low-phosphorus symptoms until the second year, while cows brought to his farm from other farms located in the same neighborhood showed the symptoms the first winter.

Results with E 121

E 121 was one of the animals used in the experiment that will be reported regarding the relation of magnesium sulfate to phosphorus deficiency. She is also included in this section of the report because after the completion of the magnesium sulfate experiment she was kept on a low-phosphorus ration until she passed through all the stages observed under farm conditions. She was finally slaughtered when she had reached a stages in which she could barely stand, and was losing weight, probably owing, in part, to a refusal to consume her feed.

Her ration during the fourteen 30-day periods in the magnesium sulfate experiment is found in Appendix Table 27. This ration consisted of low-phosphorus prairie hay; a grain mixture of corn, oats, and gluten meal; and two pounds of molasses daily, used especially as a carrier for the Epsom salt. As shown by Appendix table 27, the phosphorus intake approximated 7.5 grams per day. On this ration the inorganic phosphorus in the blood plasma showed a decline beginning the fifth month, reaching a level of 2.54 mgm. per 100 cc. during the fourteenth 30-day period. That the magnesium sulfate was not the major factor in bringing about the phosphorus deficiency, is shown by the fact that the check group receiving the same level of phosphorus intake but no Epsom salt showed even more marked results.

The feed record, mineral intake, and blood calcium and phosphorus for eight 30-day periods following the Epsom salt experiment are found in Appendix table 11. It will be noted from these data that the phosphorus intake was continued at essentially the same level until the last period, during which considerable quantities of feed were refused.

The first mild symptoms of pica appeared during the latter part of the Epsom salt experiment, when the inorganic phosphorus in the blood plasma was about 3 mgm. per 100 cc. Some indications of unthriftiness and lack of normal growth began to appear shortly after this time. The first evidence of stiffness was noted in January, 1930, after receiving the low-phosphorus ration for about 14 months. Following the replacement of a portion of the prairie hay with dried beet pulp and the elimination of the magnesium sulfate from the ration, her physical condition rapidly became worse. She showed a voracious appetite for bones and pieces of wood and began to refuse more of her feed, especially the hay, beet pulp, and molasses. The stiffness gradually became worse during the last two months and she preferred to lie down most of the time. She moved with great effort and apparently with great pain. At this stage she lost most of her desire to chew bones and wood. No gain in weight was made during the last two months. The composition of the bones of this animal is given in Tables 13, 14, and 15.

Results with E 147

When our original project concerning phosphorus deficiency in cattle was well under way its scope was enlarged to include the phosphorus requirements of cattle. This part of the project has been under way for several years, but to date the results are not complete and have not been published. The purpose is to study the effects of rations low in either calcium or phosphorus or both, and the effects of supplying these minerals in a ratio varying widely from that usually found in rations fed under practical conditions. In this study, determinations of calcium and phosphorus in the blood plasma are made monthly, and at the end of the experimental period the animal is slaughtered and the bones are analyzed.

Out of this group of animals, E 147 is selected from among several available and the data are presented as typical of heifers receiving a ration inadequate in phosphorus during the growing period. E 147 was a grade Holstein heifer, normal in size and condition at birth and until subjected to a phosphorus-deficient ration. She received the typical ration used in the University herd previous to the dates covered by the experimental data found in Appendix table 12.

At the age of 276 days she was placed in a group that was to receive a ration low in phosphorus but containing a normal amount of calcium. The blood Ca and P figures show she was normal at the end of Period 1. During this period she was still receiving a normal ration with an ample Ca and P intake. She was gradually changed to a low-phosphorus prairie hay and later a portion of this was replaced by dried beet pulp.

Low-phosphorus gluten meal was used in the grain mixture as a means of supplying the desired protein without increasing the phosphorus intake. The large variety of feeds used during the thirteen 30-day periods is accounted for by changes necessary to maintain a suitable ratio between the Ca and P and at the same time to supply the total digestible nutrients and digestible protein necessary to meet the normal growth requirements. She began to suffer severely from the phosphorus deficiency and difficulty was experienced in getting her to consume the desired amount of nutrients, which resulted in further changes in the ration. Beginning with the twelfth period, $CaCO_3$ was added to maintain the desired calcium intake and to avoid a possible complication from a shortage of this mineral. Beginning with the second period when she was first subjected to the low-phosphorus ration, the average daily phosphorus and calcium intake was as follows:

	Ca gm.	P gm.
Average daily intake	13.70	5.87
Average daily intake per 1,000 pounds body weight	23.12	8.98
Range in daily intake	9.04-24.59	4.79-7.08

The inorganic phosphorus in her blood plasma dropped to 3.12 mgm. in the second period, the first with a decidedly low phosphorus intake. A still lower level of blood phosphate soon followed that persisted throughout the 360 days of the experiment covered by the data in Appendix table 12. The figures ranged most of the time near 2.5 mgm. but dropped to a low point of 1.68 mgm. during the twelfth 30-day period. The blood calcium was normal throughout.

The first indications of pica were observed during the third period. As the experiment progressed she exhibited all the symptoms characteristic of phosphorus deficiency, including marked pica, poor appetite for hay, a decided retardation in growth except in the head, listlessness, and stiffness. A few weeks before slaughter her stiffness became so extreme that she had difficulty in getting up, and stood with the rear feet forward, the front legs spread wide apart, and the knees bent forward. At the time of slaughter it was noticed that the skull bones were thin. A considerable amount of synovial fluid was found around the hock and knee joints. The ribs indicated a lack of proper ossification by the ease with which they could be bent and broken, but no evidence could be found of enlargement at the costochondral junctions. Examination of the viscera and internal organs revealed no abnormalities.

The following description of the condition of the joints was prepared by Dr. H. C. H. Kernkamp, of the Division of Veterinary Medicine.

"The examination made was strictly confined to the gross appearance of some of the large articulations of the limbs. One of the first and noticeable lesions was the rather marked periarticular enlargement of the scapulo-humeral (shoulder) humero-radial (elbow) and femorotibial (stifle) articulation. The fibrous layer of the joint capsule in these cases was greatly thickened, as was also the synovial layer. In fact, there were some very marked villous-like proliferations of the synovial membrane into the joint cavity. These occurred mostly along the periphery of the marginal cartilage and especially was this noticed in the scapulo-humeral joint. The articular cartilages at the proximal extremity of the humeri, as well as those at the distal extremity of the femur, were grown into sort of folds, or wrinkles. The folds in some cases ran in the direction of the diameter of the joints, whereas in other places one would note the folds following the periphery of the articulation. Very definite areas of erosion and necrosis were seen in the depressions between some of the folds and more striking, perhaps, were some of the erosive lesions affecting the distal articular surface of the scapula. The cartilages were often thin and in some places denuded, so the osseus tissue could be seen quite readily. In addition to this, I felt that some of the articular surfaces were softer than normal. I mean by this that it was possible to cut into the end of the bone with the blade of a knife much more readily than in a more normal individual."

The composition of the bones of this animal is given in Tables 13, 14, and 15.

Results with Cows 59 and 82

In the course of experimental work not covered by this report, we have had occasion to feed rations that produced typical symptoms of phosphorus deficiency to a considerable number of animals. From these data the results for cows 59 and 82 are selected as representing conditions quite different from those so far presented.

Preceding the period of low-phosphorus intake the two animals were used in an experiment covering three years in which the objective was to study the relation of the calcium intake to reproduction. The ration was timothy hay and a grain mixture composed of 5 parts each of corn and barley and 3 parts each of standard wheat middlings and corn gluten meal. Cow 59 was a member of a group that received during the three years a low calcium intake; Cow 82 was in a group known as a medium calcium group that received a calcium carbonate supplement.

The ration received by both of these animals during this three-year period carried the same phosphorus content and was designed to supply a sufficient amount of this mineral. However, after the three-year experiment had been under way for some time the inorganic phosphorus content of the blood plasma indicated that the phosphorus intake was at least close to the lower limit, possibly a little below the actual requirement, as shown by a tendency for the blood phosphorus to be somewhat lower than is generally looked upon as normal. At the close of this three-year experiment, part of the animals, including No. 59 and No. 82, were put on a ration designed to be adequate in all respects except in phosphorus. The ration selected was prairie hay grown on low-phosphorus soil and containing 0.061 per cent phosphorus and a grain mixture composed of 2 parts corn, 1.5 parts corn gluten meal, and one part each of barley and dried beet pulp. The animals received as much hay as they would readily consume and sufficient grain to bring the total digestible nutrient intake to a level equal to that prescribed by Morrison's Feeding Standard (15), or slightly above.

The figures in Table 1 show the intake, milk production, and calcium and inorganic phosphorus content of the blood plasma. The preliminary period included in this table is the final 15-day period of the three-year experiment already mentioned. The data for the preliminary period, therefore, are typical of the feed intake to which these animals had been subjected over a long interval previous to the beginning of the period of low-phosphorus feeding.

Table 1

Feed Records and Blood Analyses of Two Cows Receiving a Low-Phosphorus Ration

30-day periods	Average per day					In 100 cc. blood plasma	
	Prairie hay	Grain mixture	Milk produced	Ca intake	P intake	Ca	P
	lb.	lb.	lb.	gm.	gm.	mgm.	mgm.
Cow 59							
Preliminary*	10.0	15.0	30.1	21.30	31.45	9.89	4.98
Period 1	18.6	11.5	27.0	26.62	16.58	10.33	1.33
Period 2	20.4	9.5	24.3	50.73	15.35	10.48	1.93
Period 3	20.8	9.0	22.2	51.26	14.95	10.60	1.10
Period 4	20.2	7.9	19.9	50.91	15.00	10.88	1.15
Cow 82							
Preliminary*	7.7	16.0	38.5	20.83	32.97	9.51	5.35
Period 1	15.7	14.5	38.4	42.06	18.85	10.33	1.46
Period 2	20.9	9.9	32.1	50.28	19.38	10.59	1.08
Period 3	20.1	10.7	31.6	47.38	24.03	10.53	1.24
Period 4	20.3	10.2	29.5	47.10	22.71	10.18	0.52

* During this period the hay was timothy and the grain mixture was corn, barley, wheat middlings, and corn gluten meal.

It is desirable to have in mind the previous history of these animals because of its importance in explaining, in part, the remarkable decline in the inorganic phosphorus content of the blood as shown in Table 1, and the early development of pica. The two animals selected for presentation were about the mean of a group of seven, two of which showed an even more extensive decline in blood phosphorus.

The extremely rapid decline in the blood phosphorus was due to three causes. First, owing to the previous treatment of the animals over a three-year period, the reserve of phosphorus in the body was unquestionably low, as shown by a tendency for the blood phosphorus to be slightly below normal. The second factor was milk production by both cows, which causes a heavy drain on the body. It is noticed under farm conditions, as well as under experimental control, that the first cases and the most severe ones occur among growing animals and cows in milk. The third factor was the very low phosphorus intake. Armsby's figures for the phosphorus requirements of maintenance and milk production indicate a necessary intake for Cow 59 of approximately 30 grams of phosphorus daily, or about double the amount she actually received. Cow 82, on the same basis, also should have received approximately double her actual intake.

In the group to which these animals belong, the first symptoms of pica shown by a desire to chew bones were observed after about two months on the low-phosphorus ration. Later, lessened appetite for hay was experienced.

BLOOD PHOSPHORUS STUDIES

In 1919 Meigs, Blatherwick, and Cary (16) reported variations in the inorganic phosphorus in the blood plasma of lactating cows corresponding with a decrease or increase in the proportions of grain and hay fed in a grain-hay-silage ration. They reported further that the feeding of sodium phosphate to heifers increased the inorganic phosphorus of the blood plasma. Of two cows studies, one showed a decrease in phosphate concentration from 5.6 to 4.6 mgm. per cent and the other from 7.7 to 4.6 mgm. per cent after about 35 days of reduced hay and grain. The blood phosphate of one heifer increased from 6.3 to an average of 8.4 mgm. per cent after feeding from 4.5 to 6.9 grams phosphorus daily as NaH_2PO_4.

Jones and Mullen (17) observed a tendency of the inorganic phosphorus in the blood plasma of cattle to rise when bonemeal or raw phosphate rock was added to the ration, with a tendency to fall again when the phosphate supplement was withdrawn; but Robinson and Huffman emphasize the fact that the variations noted were within the normal range of fluctuation of inorganic phosphate on a constant diet.

We obtained the first indication of abnormally low blood plasma phosphate in connection with the phosphorus deficiency disease of cattle in June, 1926. Samples of venous blood taken from the jugular vein of E 90 on June 8, 9, and 10 showed plasma phosphate concentrations of 1.8, 1.6, and 1.6 mgm. per cent, respectively. This animal was a mature, lactating cow. She had been brought to the station in May, 1926, from the phosphorus deficient area of Minnesota as a good representative of a badly affected animal. She was very thin and stiff, and her joints creaked when she walked. She also showed strong craving for sticks and bones. Striking proof was soon obtained of the importance of the blood phosphate concentration as an index of phosphorus deficiency in cattle and as a direct physiological causative agent in the disease. These data, which are shown in Table 2, were presented in December, 1926, and published by Palmer and Eckles (2) in January, 1927. The analyses represent the average of samples of jugular venous blood taken on three successive days in the months shown. All the animals had been reared in the phosphorus-deficient region of Minnesota and had been under experimental observation at the Station for at least 75 days when the first analyses were made. The hay fed had been grown in the deficient area. It was fed *ad libitum*. In addition, oats were fed at the rate of 2 to 12 pounds daily, per head, depending on whether the cattle were dry or milking and on the amount of milk produced. The data show clearly the abnormally low inorganic phosphorus in the blood plasma of the animals confined to the hay-oats ration, either with or without $CaCO_3$ supplement. The total plasma Ca, however, was normal. In contrast is the normal composition of the blood when sodium phosphate was fed.

Table 2

Effect of Phosphorus Deficiency and Phosphorus Feeding on the Inorganic P and Total Ca of the Blood Plasma of Dairy Cattle

Animal No.	Mineral supplement fed	Period of feeding mineral*	Inorganic P and total Ca in 100 cc. blood plasma during											
			August			September			October			November		
			P	Ca	CaxP	P	Ca	CaxP	P	Ca	CaxP	P	Ca	CaxP
			mgm.	mgm.		mgm.	mgm.		mgm.	mgm.		mgm.	mgm.	
E 33†	None	...	2.55	12.29	31.2	2.20	13.46	29.6	2.99	12.51	37.4	1.75	12.81	22.4
E 73	None	...	2.47	12.13	30.0	2.11	13.49	28.5	2.28	12.87	29.2	1.99	12.25	24.4
E 91†	None	...	2.39	11.87	28.3	1.93	12.82	24.7	1.26	11.78	14.5	1.03	11.60	11.9
E 94†	None	...	1.28	13.01	16.6	2.04	15.20	31.1	1.32	16.02	21.1	1.09	10.97	11.9
E 75	$CaCO_3$‡	102	1.75	12.19	21.3	1.80	14.63	26.3	1.94	13.30	25.8	2.38	12.62	30.0
E 93†	$CaCO_3$‡	73	1.07	12.06	12.9	2.02	14.60	29.6	1.03	16.47	16.9	1.58	13.17	20.8
E 58	NaH_2PO_4§	600	6.56	11.29	74.1	5.43	13.11	71.2	...	....	...	...	....	...
E 74	NaH_2PO_4	102	5.51	10.65	58.7	5.17	11.78	60.9	5.40	11.63	62.8	4.75	10.53	50.0
E 92†	NaH_2PO_4	85	4.41	12.66	55.8	3.82	12.86	49.1	5.27	12.06	63.6	6.04	10.51	63.5

* Up to time of first analysis.

† Cow in milk.

‡ 100 grams daily for cows 75 and 93.

§ 75 to 100 grams daily for cows 58, 74, and 92. The compound fed had the composition $NaH_2PO_4 \cdot H_2O$.

Since these data were first published, similar results for whole blood have been reported by Theiler, Green, and du Toit (18) and by Malan, Green, and du Toit (19) for cattle in the phosphorus-deficient area of South Africa.[4] Schermer and Hofferber (20) have obtained similar data for cattle in Germany suffering from osteomalacia and Knockenweiche (soft bones).

Since August, 1926, it has been the practice at this Station to make frequent determinations of the concentration of inorganic phosphorus and total calcium in the blood plasma of all the cattle on the various phases of the phosphorus-deficiency problem. These have sometimes been made daily or every other day, sometimes at fortnightly or bimonthly intervals, but the largest number have been at monthly intervals. Nearly all the fortnightly, monthly, and bi-monthly analyses have been made on a composite sample of equal volumes of plasma obtained on three successive days. As the result, a very large amount of confirmatory data has been secured on the importance of the blood composition in diagnosing the various stages of the prosphorus-deficiency disease. These data are much too numerous to report in detail. Certain aspects of the study are of special interest, however, and representative data will be presented to illustrate certain of the relationships found.

Two aspects of the problem of phosphorus-deficiency disease in cattle of particular significance are the rapidity with which animals recover when phosphate supplements are administered and the rapidity with which the disease appears when inadequate amounts of phosphorus are ingested. The blood phosphate concentration is a very significant index of these conditions.

Rate of Recovery of Blood Phosphate

The data in Table 3 illustrate the rapidity with which three animals, E 93, E 94, and E 75 recovered. These animals had been under experimental observation on the phosphorus-deficient prairie hay-oats ration for at least 200 days before the phosphate supplements were begun. The animals were brought to the Station from the phosphorus-deficient area in May, 1926. The marked symptoms exhibited at that time had become intensified during the period prior to the time the phosphate feeding was begun. The blood phosphate concentration was restored to normal in 5 to 8 days in the case of E 75 and E 94, and in 17 days in the case of E 93. Two types of recovery are seen, namely, (1) a very rapid building up of surplus amounts of plasma phosphates which

[4] The South African workers reported in all the analyses of 42 blood samples from 39 animals (12 dry cows, 12 lactating cows suckling calves, and 15 heifers 18 to 20 months old) suffering from varying degrees of phosphorus deficiency while grazing on the veldt, and a somewhat greater number of analyses from animals fed a supplement of bonemeal. All analyses were on the whole blood. The concentrations of inorganic phosphate reported, which averaged 1.9 (range 1.0 to 3.6) mgm. per 100 cc., are not directly comparable to our data based on the plasma, but are about 40 per cent too low if it be assumed that the plasma constitutes on the average about 60 per cent of the blood volume. Inorganic phosphate is practically absent from the blood corpuscles. The South African data converted into plasma values on this basis would average 3.2 mgm. per cent of inorganic phosphate.

were subsequently absorbed by the body tissues so that the phosphate concentration fell to a more normal level; and (2) a less rapid building up of normal phosphates in the blood plasma without evidence of the high temporary concentrations. It is not possible to explain this difference with the evidence at hand, inasmuch as E 75 and E 93 had been on the same ration previous to the phosphate feeding, both having received 100 grams $CaCO_3$ daily for about 200 days prior to the substitution of this compound by $Ca_3(PO_4)_2$. The feeding of the more soluble phosphate, $NaH_2PO_4 \cdot H_2O$, might have been expected to exert the effect noted.

The rise of inorganic phosphorus in the blood in all cases was accompanied by some decrease in total calcium, but the calcium remained within normal limits at all times, showing that calcium deficiency was not involved. This is particularly true for E 94, which received no calcium supplement in the preliminary period.

Table 3
Effect of Phosphate Supplements on Rapidity of Recovery of Blood Plasma Phosphate

Animal	Date	Plasma inorganic phosphorus	Total plasma calcium	Daily phosphorus supplement	Symptoms
		mgm. per cent	mgm. per cent	gm.	
E 93	12/20-22/1926	1.00	13.07		Intense pica.
	1/11/1927	2.12	12.92	100 g. $Ca_3(PO_4)_2$ begun 1/9/27	Condition very poor.
	1/16	2.84	11.30	"	....
	1/21	3.05	12.35	"	Animal had gained 50 lbs.
	1/26	5.28	12.35	"	Since 12/23. No pica.
	1/31	4.90	12.35	"	....
	2/14	5.18	11.92	"	....
	2/21-23	5.02	11.73	"	Further gain of 40 lbs.
E 94	12/16/1926	0.77	13.68	....	Strong pica.
	12/22	1.27	12.73	100 g. $NaH_2PO_4 \cdot H_2O$ begun 12/22/26	....
	12/27	5.10	10.64	"	....
	1/1/1927	8.19	9.88	"	....
	1/6	6.42	9.97	"	....
	1/11	7.09	12.59	"	....
	1/23	5.03	10.49	"	Condition greatly improved.
	1/26-27	6.19	11.40	"	....
	2/21-23	5.46	11.21	"	....
E 75	12/16/1926	1.75	12.92	....	Marked pica.
	12/22	2.46	12.73	100 g. $Ca_3(PO_4)_2$ begun 12/22/26	....
	12/27	4.76	10.54	"	....
	1/1/1927	8.13	9.64	"	....
	1/6	5.40	11.11	"	....
	1/11	6.80	12.78	"	....
	1/23	5.40	11.97	"	Animal had gained 20 lbs.
	1/26-27	5.33	12.63	"	General condition much improved.
	2/21-23	5.55	11.87	"	....

The effect of the phosphorus supplements on the general condition and physiological behavior of these animals was striking. Whereas they had been exhibiting marked to intense desire for bones and sticks for months prior to the feeding of the phosphates, this disappeared almost immediately. E 75, and especially E 93, began to gain in weight, the latter gaining nearly 100 pounds in the course of the first 40 days of $Ca_3(PO_4)_2$ feeding. E 94 did not gain in weight because she freshened within a month after phosphate feeding began. All the animals improved markedly in general physical condition and appearance. This was very rapid and was simultaneous with the rise in blood phosphate. The importance of an adequate concentration of inorganic phosphorus in the blood for general metabolic welfare is very strikingly shown by these experiments.

Another striking illustration of rapid blood phosphate recovery on administration of $NaH_2PO_4 \cdot H_2O$ to severely affected animals is shown in Figure 1. The curves show the rise in both total and inorganic phosphorus of the blood plasma of E 150 and E 153. These animals were

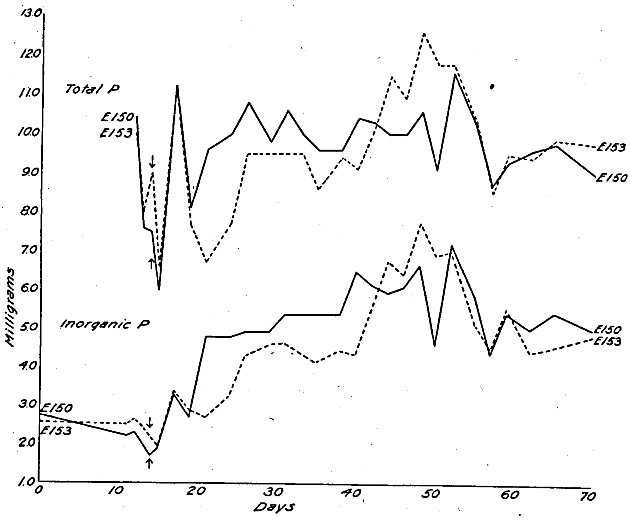


Fig. 1. Total and Inorganic Phosphorus in the Blood Plasma Before and After Feeding $NaH_2PO_4 \cdot H_2O$
Vertical arrows indicate the beginning of phosphate feeding.

purchased in the phosphorus deficient area of Minnesota in May, 1930, and when brought to the Station were in very poor condition and exhibited all the symptoms of advanced phosphorus deficiency. They were continued on a low-phosphorus ration for a short time before the feeding of phosphate was begun. $NaH_2PO_4 \cdot H_2O$ amounting to 21 grams (equal to 4.7 grams of phosphorus) was fed daily. This was continued for 57 days. As in the experiments with E 93 and E 94, recovery of normal blood phosphate concentration occurred within two weeks. Also as in the case of the sodium phosphate feeding to E 94, there was a building up of phosphate concentration followed by a decline to normal values.

The data regarding the changes in total phosphorus in the plasma are of special interest in showing that in general these followed fairly closely the changes in inorganic phosphate. This result indicates that the inorganic phosphates are the active forms in the blood plasma concerned with mineral nutrition. The selection of these forms for diagnosis of phosphorus deficiency disease is, therefore, significant as well as fortunate.

As has been previously pointed out, the rate of advance of phosphorus deficiency in cattle depends on various combinations of several conditions, namely, the level of phosphorus intake, the level of calcium intake, the age of the animal, its previous nutritive condition, and whether or not the animal is lactating. The lower the level of phosphorus the more rapid the progress of the disease, and this is accelerated by a high calcium intake, by low reserves of phosphorus, and by lactation. In all cases, however, the plasma phosphate concentration has been found to be an important index that the animal is suffering from phosphorus deficiency. Data illustrating some these facts are presented in Tables 3, 4, and 5.

Rate of Fall of Blood Phosphate

The data in Table 4 show the relative rapidity with which the blood phosphate falls at different levels of phosphate deficiency, other factors, such as mineral reserves, milk production, etc., being essentially equal. Both animals had ample mineral reserves and their milk production, as seen in the table, was about the same. Cow E 74, however, on a ration of prairie hay and oats showed only a slow decline in blood phosphate, a concentration indicating deficiency not being reached for five to six months. On the other hand, Cow 68, fed a ration lower in phosphorus, being made of timothy hay and grain, up to November 1st when it was changed to the low phosphorus ration of prairie hay, beet pulp, and grain, showed a marked drop in blood phosphate within four weeks.

A noteworthy fact brought out in this comparison is that a rapid fall of blood phosphate to apparent deficiency levels is not accompanied by the simultaneous appearance of pica. Pica appears gradually, Cow 68, for example, beginning to show pica only after her blood phosphate concentration had remained at a deficiency level for about three months. This fact appears to indicate that the pica of phosphorus deficiency is not the direct result of low blood phosphate but is a symptom associated with a more general metabolic disturbance accompanied by blood phosphate deficiency.

Table 4
Fall of Blood Phosphate in Moderate and Severe Phosphorus Deficiency in Milking Cows

Cow	Weight*	Average daily milk†	Average daily P intake per 1,000 lb. body weight‡	Date of blood analysis	Plasma inorganic P	Plasma Ca
	lb.	lb.	gm.		mgm. per 100 cc.	mgm. per 100 cc.
E 74	995	16.3	33.8	3/21-23/1927	4.66	10.45
	946	21.3	37.6	4/20-22	3.72	11.21
	987	19.6	39.5	5/21-23	2.70	10.21
	1,001	18.4	19.0	6/27-29	4.16	11.23
	1,001	16.0	18.5	7/20-22	3.82	11.12
	999	16.2	13.7	8/20	...	....
	988	13.1	13.2	9/15-17	1.64	11.55
Cow 68	1,191§	17.0§	18.5§	10/27-29/1931	4.12	10.48
	1,221	16.5	12.6	11/24-26	2.67	10.25
	1,202	14.9	10.1	12/28-30	2.07	10.65
	1,187	14.7	10.2	1/26-28/1932	2.19	10.33
	1,191	14.4	10.9	2/23-25	1.54	10.55

* Weight at end of a 30-day period near or at the end of which the blood analyses were made.

† Based on the milk production for the 30-day period at the end of which the weight shown was taken.

‡ Based on food consumed and its P analysis for the 30-day period for which the average milk production is calculated.

§ First period for Cow 68 was 15 days.

Table 5
Influence of Lactation on Rate of Fall of Blood Phosphate in Phosphorus Deficiency

Cow	Weight*	Average daily milk†	Average daily P intake per 1,000 lb. body weight‡	Date of blood analysis	Plasma inorganic P	Plasma Ca
	lb.	lb.	gm.		mgm. per 100 cc.	mgm. per 100 cc.
E 92	871	29.1	50.2	3/28-30/1927	5.04	11.40
	968	25.5	26.8	4/20-22	2.31	12.16
	979	24.4	20.0	5/21-23	2.04	11.02
	955	22.5	20.4	6/27-29	1.58	12.09
	955	18.8	17.1	7/20-22	1.53	11.55
	916	16.1	13.5	8/20	...	....
	898	11.9	15.1	9/15-17	1.17	12.96
	940	17.2	12.1	10/18-20	2.67	12.74
E 151	728	...	19.6	7/5, 7, 9/1930	6.04	....
	722	...	18.9	8/1, 4, 6	5.50	....
	767	...	17.2	9/2-4	6.60	11.38
	786	...	17.4	10/3-5	7.14	11.15
	823	...	16.8	11/3-5	5.43	12.06
	847	...	16.4	12/2-4	4.82	10.83

* Weight at end of a 30-day period near or at the end of which the blood analysis was made.

† Based on the milk production for the 30-day period at the end of which the weight shown was taken.

‡ Based on food consumed and its P analysis for the 30-day period for which the milk production was calculated and body weight taken.

Influence of Milk Production

The data in Table 5 need very little comment. They show in a very striking way to what extent milk production is a strain in phosphorus deficiency and decreases blood phosphate, whereas a similar phosphorus intake by a dry cow permits the maintenance of blood phosphate values and normal physical condition over several months. Animal E 151 was, however, beginning to show a slight decline in blood phosphate toward the close of the last period shown in Table 5.

Table 6
Influence of Calcium Intake on Rate of Fall of Blood Phosphate in Phosphorus Deficiency

Date* 1930-31	Cow 150: Daily mineral intake per 1,000 lb. body wt.† Ca	Cow 150: Daily mineral intake per 1,000 lb. body wt.† P	Cow 150: Plasma inorganic P‡	Cow 151: Daily mineral intake per 1,000 lb. body wt.† Ca	Cow 151: Daily mineral intake per 1,000 lb. body wt.† P	Cow 151: Plasma inorganic P‡
	gm.	gm.	mgm. per cent	gm.	gm.	mgm. per cent
12/2/30	50.5	9.8	4.10	170.2	9.6	4.82
1/1/31	46.0	9.1	3.72	167.2	9.1	3.44
1/31	39.1	9.3	2.97	187.9	9.6	2.82
3/2	38.6	9.7	2.70	195.7	9.8	2.49
4/1	38.6	10.0	3.62	185.6	10.3	2.89
5/1	36.2	10.6	4.35	194.0	10.6	3.31
5/31	33.6	9.9	4.35	186.0	9.9	3.45
6/30	33.0	9.5	3.82	176.8	9.5	2.98
7/30	31.1	9.1	3.70	171.5	9.3	3.66
8/29	30.8	8.9	3.52	170.6	9.3	2.64
9/28	27.0	8.4	3.13	164.4	8.8	1.53
10/28	25.8	8.0	2.62	156.6	8.1	1.26
11/7	24.6	7.5	2.62	154.7	7.6	1.26

* Thirty-day periods ending on date given.
† Average daily intake calculated from food consumed and the mineral composition of the food, determined by analysis.
‡ Composite sample of plasma from three successive days near the middle of the period.

Influence of Calcium Intake

In the experiment shown in Table 6, in which an excess of calcium was fed to E 151, the effects of the added calcium were very striking, on both the blood composition and the symptoms of phosphorus deficiency. Both animals weighed about 800 pounds at the beginning and gained about 200 pounds during the eleven months' feeding. The consumption of nutrients was practically the same, and both animals showed lack of appetite for hay most of the time. The aim was to allow a phosphorus intake a little under normal. Judged from the gradual decline of the inorganic phosphorus in the plasma of E 150 during the latter part of the period, a mild phosphorus deficiency was present. It was not severe enough in the case of this animal, however, to affect her appearance or cause any indications of pica. On the other hand, E 151, which ingested the same amount of phosphorus, became more or less rough and unthrifty in appearance and exhibited pica almost constantly. Data on total blood plasma calcium taken simultaneously with the inorganic phosphorus data shown in Table 6, but not included in the table, showed normal values throughout.

Use of Blood Analysis in Field Diagnosis

The marked effect of phosphorus deficiency on plasma phosphate concentration suggested the use of blood analysis as a means of diagnosing this condition in the field. Green and Macaskill, in South Africa (21) have employed it for this purpose. Opportunity to do this at this Station has been somewhat limited, but the few data available are shown in Table 7. If the plasma inorganic phosphorus is to be employed, as has been done in all the controlled experiments at this Station, a technical difficulty is presented by the fact that the plasma must be prepared almost immediately after the blood sample is taken in order to prevent a breakdown of the organic phosphorus of the blood corpuscles. Any decomposition of these organic compounds, which comprise a large proportion of the total phosphorus of the whole blood, gives rise to abnormal values for plasma phosphate. This difficulty is not serious in the hands of a competent veterinarian who understands the situation and takes proper precautions. However, several samples sent in to the Station have not been suitable for inorganic phosphate determinations.

The data in Table 7, from the herd of owner N. B. T., represent samples taken in the field by one of the Station assistants. The condition that existed on this farm is of particular interest because the owner was attempting to avoid phosphorus deficiency by the use of bonemeal, altho pica appeared in spite of this precaution. His farm is located in one of the most phosphorus-deficient areas in Minnesota. Not only do the blood analyses in Table 7 show why most of his cows tested were exhibiting pica but analyses of his pasture and timothy hay, even that grown in phosphate-treated soil, explain the occurrence of pica in spite of bonemeal feeding. Two samples of pasture, one upland and the other lowland, contained 0.063 and 0.072 per cent phosphorus, respectively (air dry basis), and two samples of timothy hay, one of untreated and the other from phosphate-fertilized soil, contained 0.052 and 0.072 per cent phosphorus. With these very low-phosphorus products making up the bulk of the ration of these milking cows, and with an estimated bonemeal consumption of only one pound per week per head (equivalent to a daily phosphorus intake of 17.5 grams, based on actual analysis of the bonemeal being fed), it is evident that the phosphorus consumption was insufficient to prevent phosphorus deficiency from developing. It was recommended that the bonemeal be increased to 2 pounds per head per week. No further complaints being received, it may be assumed that the pica disappeared.

The sample from Farm 2 was said to be from a cow that was so stiff and lame that she was unable to get up; several other cows in the herd were similarly affected. The plasma phosphate obtained on analysis is probably too high for an animal in the condition described, but this is explained by the fact that the sample showed evidence of being lakey. A sample of hay from this farm contained only 0.086 per cent phosphorus. The farm is in the phosphorus-deficient region of Minnesota.

Table 7

Inorganic P in Blood Plasma of Cattle in Field Cases of Phosphorus Deficiency

Farm	Date	Owner	Cow	Inorganic P in plasma	Total Ca in plasma	Symptoms
				mgm. per cent	mgm. per cent	
1	August, 1929........	NBT	Spot	1.47*	11.03	Pica
	"	"	Kate	2.92	8.91	"
	"	"	Signe	3.17	9.76	"
	"	"	Kicker	3.50	10.16	"
	"	"	Ruth	3.78	10.21	"
	"	"	.....	4.35	9.82	Normal
2	March, 1930........	GW	.....	3.26†	....	Lame and stiff

* All samples from farm of N. B. T. brought in by Station assistant.

† Sample sent in by local veterinarian. Hay from this farm contained only 0.086 per cent phosphorus. Blood sample not in good condition when received, so phosphate value is probably too high.

Altho the data in Table 7 are limited, they show clearly the value of blood analysis as an aid in diagnosis of phosphorus deficiency in cattle, especially if coupled with analyses of hay or pasture from the affected farm.

Normal Variations in Blood Phosphate

During the collection of the large amount of blood data, some of which are here reported, unaccountable and frequently large fluctuations in the concentrations of inorganic phosphate were observed to occur from day to day. It was this finding that led to the adoption, as far as possible, of the mean of the analytical values obtained on three consecutive days' samples as the probable true value. Robinson and Huffman (22) noted similar fluctuations for the blood of beef cattle, and Havard and Reay (23) observed the same fact for human blood.

The problem raised by these findings is of considerable general interest as well as of importance in the diagnosis of phosphate deficiency by blood analysis and in the employment of such data for determining the phosphorus requirements of cattle.

The general aspects of the problem are in connection with the effects on the blood phosphate concentration of various physiological factors such as exercise, water drinking, maturity, and parturition. Some of these problems have been studied with other species of animals and in a few instances with cattle. For example, Meigs, Blatherwick, and Cary (16) noted that the inorganic phosphate in cow's blood is likely to be low just after calving, and that of the calf considerably higher. Under normal conditions of nutrition, the blood phosphate of calves is shown to rise to a maximum at about the age of six months and then gradually fall to somewhat lower levels for mature animals. Robinson and Huffman (22), in general, confirm the results of Meigs and associates for the effects of parturition, but note further that the low value of plasma phosphate lasts for only a few hours after parturition, rising to a rather definite maximum during six to eighteen hours after calving, with a subsequent depression. An extensive study of the partition of phosphorus in the blood of calves and their dams is reported by Green and Macaskill (21).

Experiments conducted at this Station (6) have shown the following results in studying some of the physiological variations previously mentioned:

Exercise causes marked changes in blood phosphate of cattle.

Feeding has a small but significant effect on the inorganic phosphate in the blood of cattle. The value rises within the first hour and apparently does not return to normal until after about three hours.

Normal water drinking by cattle has no significant effect on blood phosphate.

So far as the above factors are concerned, the procedure indicated for securing normal samples of blood from cattle is to have the animal at rest in its stanchion for several hours and draw the blood before feeding, with the least possible physical disturbance of the animal. Water should be allowed *ad libitum*.

Parturition causes a decrease in the inorganic blood phosphorus, which may amount to as much as 3.2 mgm. per 100 cc. of plasma. The decrease sets in on the day before calving, the lowest point of decrease occurring either before or after parturition.

The phosphate content of the blood of a calf at birth is higher than that of the dam, and compares favorably with the value shown by the dam several days before calving.

The inorganic phosphorus content of the blood of calves increases until about six months old, after which it decreases until the normal range for mature cattle is attained.

The day to day or hour to hour fluctuations in plasma phosphate are of special importance for blood sampling when the composition is to be employed for diagnostic purposes. It has been found at this Station that the fluctuations in inorganic phosphate concentration may be relatively great, i.e., as much as 15 to 25 per cent, at two-hour intervals during the day. The day to day fluctuations in periods of three successive days showed that about 75 per cent of the samples taken on the second and third days varied significantly, i.e., more than 10 per cent, from the expected value. A set of sixty three-day periods was employed for this study.

Altho the data in Tables 2 to 7 and Figure 1 show clearly the general effect of phosphorus intake on the concentration of inorganic phosphate in the blood plasma, the mathematical relationship between these values is not sufficiently close to enable one to employ them as the sole criteria for determining the phosphorus requirements of cattle. A statistical study has been made of the coefficient of correlation between plasma phosphate concentration and phosphorus intake per unit of body weight of 10 dry cows, representing 56 observations, used in the phosphorus-deficiency studies. The phosphorus intake in these cases varied from 5.4 to 12.4 grams daily per 1,000 pounds body weight, and the inorganic phosphorus in the blood plasma from 1.65 to 5.33 mgm. per 100 cc. The correlation found is $r = + .157 \pm 0.88$, which is not significant.

BONE COMPOSITION IN PHOSPHORUS DEFICIENCY

The majority of the local terms employed in various parts of the world to describe the symptoms of phosphorus-deficiency disease in cattle include some reference to bone abnormalities. Such terms as Knochenbruchigkeit (bone-breaking) or Knochenweiche (bone-softness) in Germany, stifziekte or stijwesiekte (stiff-sickness) in South Africa, stiffs or cripples in Australia and New Zealand illustrate the terminology used to describe various aspects of the more generalized term "osteomalacia." Altho these references to bone pathology show that it is one of the major effects of phosphorus-deficiency disease, no studies seem to have been made on the chemical composition of the bones in cattle thus affected except those either made at this Station or initiated by it.

Becker and Neal (24) have published the results of Becker's studies on the breaking strength of the bones of three animals brought to the

Station from the phosphorus-deficient area of Minnesota and of one animal that had been cured by feeding sodium phosphate. These data are shown in Table 8.

Table 8

Breaking Strength of Bones from Phosphorus-Deficient Cattle in Comparison with Those of a Cured Animal

Animal	Ration	Supplement	Bone broken	Span	Breaking strength Left	Right	Average
				in.	lb.	lb.	lb.
Daisy	P-deficient	none	rear cannon	9	1,750	1,270	1,510
			6th rib	9	100*	80*	90*
E 34	P-deficient	$NaH_2PO_4 \cdot H_2O$	humerus	6	4,050	....	4,050
		246 days	femur	9	3,200	....	3,200
			6th rib	9	190*	....	190*
			11th rib	9	130*	....	130
E 36	P-deficient	$CaCO_3$	humerus	9	2,860	....	2,860
		334 days	femur	7	2,510	....	2,510
			6th rib	9	80*	....	80
			11th rib	9	120*	150*	135
			11th rib	7	....	150*	135
E 37	P-deficient	$CaCO_3$	humerus	9	2,280	....	2,280
		334 days	femur	7	2,350	....	2,350
			6th rib	9	150*	....	150
			11th rib	9	130*	....	....
			11th rib	7	....	150*	140

* Ribs flexible; no clean break.

The abnormally low breaking strength and the recovery by feeding phosphate are clearly shown.

Theiler (25) has reported his examination of the histo-pathology of the bones of South African cases of "stiff-sickness" in which he asserts that the osteomalacia is primarily rickets. He describes a condition of atrophy of osseous tissue present in decreasing severity as the age of the afflicted animal advances, accompanied in all cases by abnormal amounts of osteoid. The latter is a specific characteristic of rickets as seen in very young animals. Theiler regards the pathology of straight aphosphorosis of cattle and human rickets as completely identical, the diseases differing only in etiology. This difference is explained on the grounds that human rickets is a straight vitamin D deficiency, while cattle rickets is a phosphorus deficiency. He holds that the Ca:P ratio of the diet plays no important rôle in the cattle disease. He believes that the cattle in which he produced the phosphorus-deficiency disease experimentally in South Africa had ample supplies of vitamin D because of the brilliant sunshine and high altitude (4,000 feet) where the cattle were stationed.

Certain of Theiler's views seem to be open to question, inasmuch as injections of irradiated ergosterol were found by Schermer and

Hofferber (20) to improve the condition and restore to normal the blood phosphate of cattle suffering from Knockenweiche, in Germany. These authors admit, however, that complete recovery was not secured without addition of sufficient clover or alfalfa hay. Furthermore, the data presented in Table 6 in connection with the blood studies at this Station show that the Ca:P ratio of the ration may be an important factor in the phosphorus-deficiency disease of cattle.

Henderson and Weakley (26), in a study initiated at this Station in connection with this problem, have examined the general composition of the ribs, the femur, and the humerus of heifers after six and twelve months' feeding of rations that permitted the ingestion of 14.3 grams of P daily per 1,000 pounds body weight. The animals were placed on this ration at 12 to 13 months of age. The average results obtained expressed as percentage of a normal group at the same ages are shown in Table 9. Two animals are involved in each average. Only one of the four animals developed stiffness and the reported inorganic phosphate in a three-day composite of plasma shows that the deficiency developed was not severe, the lowest value reported being 4.69 mgm. per 100 cc.

Table 9

Effect of Low Phosphate Rations on General Composition of Bones of Cattle (Data of Henderson and Weakley)

Constituent	Duration of feeding	Composition of ribs	Composition of femur and humerus
	months	per cent of normal	per cent of normal
Moisture	6	106.2	105.2
"	12	117.2	116.9
Ether-alcohol extract	6	107.7	102.6
" "	12	136.1	108.1
Non-extractable organic matter	6	100.3	100.9
" " "	12	92.4	92.6
Ash	6	94.5	99.8
"	12	87.9	90.1
Calcium	6	97.2	100.1
"	12	88.7	91.0
Phosphorus	6	93.2	99.9
"	12	88.1	89.8
Ash (dry extracted)	6	98.5	99.6
" " "	12	98.0	98.5
Calcium in ash	6	101.4	100.2
" "	12	100.8	100.8
P in ash	6	98.5	99.0
"	12	100.0	99.8

In spite of the relatively moderate extent of the phosphorus deficiency, the data in Table 9 show that there was an appreciable reduction of ash in the green bones, this being replaced by water and lipid substances. The mineral content of the bones on the dry, extracted basis, was only slightly below normal. In this respect these bones do not resemble true rickets for it has been amply demonstrated by numerous investigations that the outstanding characteristic of the rachitic bone of young animals and infants is a great reduction in the percentage of ash in the dry extracted portion of this tissue.

The normal Ca:P ratio of the bones in the experiments of Henderson and Weakley, shown in Table 9, is typical of numerous studies (*27, 28, 29, 30*) of rachitic bones. For this reason additional criteria were sought that could be applied to the cattle bones in phosphorus deficiency. Howland, Marriott, and Kramer (31) and Kramer and Shear (32) have found significant variations in the composition of calcification by analyzing unashed samples of dry, extracted bone tissue. They reported the calcium phosphate : calcium carbonate ratio for the bone of normal rats to be 10.6 and 10.8, and for a rachitic rat 6.7. A similar reduction of the ratio was observed in rachitic bones of infants. This ratio is calculated from the percentages of Ca, P, Mg, and CO_2 in the dry extracted bone powder by calculating the Mg to be present as $Mg_3(PO_4)_2$ and the CO_2 to be present as $CaCO_3$. The methods of preparation and analysis of cattle bones according to this procedure, as worked out at this Station, have already been published (12). The femur and humerus and the sixth and eleventh ribs on the right side of the body are each analyzed and the mean value for $Ca_3(PO_4)_2$: $CaCO_3$ of the four bones taken to represent the composition of the mineral portion of the skeletal tissues.

Table 10

Mean Composition of Fresh Bones of Normal Dairy Cattle

Animal	Age	Ash	Alcohol-ether extract	Residue*	H_2O
	months	per cent	per cent	per cent	per cent
E 136	6	37.1	13.4	23.6	25.9
E 137	6	35.2	16.1	22.2	26.5
E 132	7	34.6	16.2	20.6	28.6
E 114	7	33.0	18.9	19.4	28.7
B 163	8	32.9	11.9	21.1	34.1
E 86	21	37.2	19.6	20.7	22.5
E 78	24	39.9	22.5	21.8	16.8
E 98	24	40.5	22.4	20.3	16.8
Average	13	36.3	17.6	21.2	25.0

* 100 — (ash + alcohol-ether extract + water).

Preliminary results already published (*7*) for bones of cattle suffering from severe phosphorus deficiency in Minnesota and for the bones from the four heifers in the experiments of Henderson and

Weakley (26), summarized in Table 9, showed an average $Ca_3(PO_4)_2$: $CaCO_3$ of 5.30 for four Minnesota specimens and an average of 6.67 for the four West Virginia animals, in contrast to an average ratio of 7.11 for normal animals 18 to 24 months of age and a ratio of 6.57 for normal mature cattle over four years of age. Since the West Virginia cattle were in the 18 to 24 months age range and the four Minnesota cattle were all mature, the relative reduction in the $Ca_3(PO_4)_2$:$CaCO_3$ ratio in comparison with the normal for the same age shows a much more severe deficiency for the Minnesota cattle and bears out the other data showing the moderate degree of deficiency induced in the West Virginia experiments.

Table 11

Mean Composition of Dry Bones of Normal Dairy Cattle

Animal	Age	Ash	Alcohol-ether extract	Residue*
	months	per cent	per cent	per cent
E 136	6	49.3	19.5	31.2
E 137	6	47.7	22.3	30.0
E 132	7	48.3	23.0	28.7
E 114	7	46.5	25.5	28.0
B 163	8	49.9	17.8	32.3
E 86	21	48.3	24.5	27.2
E 78	24	47.8	26.2	26.0
E 98	24	48.9	28.3	22.8
Average	13	48.3	23.4	28.3

* 100 — (ash + alcohol-ether extract).

Table 12

Mean Composition of Dry Extracted Bones of Normal Dairy Cattle

Animal	Age	Organic residue and minor minerals	Total ash	$Ca_3(PO_4)_2$	$CaCO_3$	$\frac{Ca_3(PO_4)_2}{CaCO_3}$
	months	per cent	per cent	per cent	per cent	per cent
E 136	6	40.03	61.2	51.5	7.57	6.82
E 137	6	40.44	61.4	52.3	7.26	7.19
E 132	7	38.94	63.0	53.5	7.56	7.08
E 114	7	41.24	62.8	51.5	7.26	7.10
B 163	8	41.71	61.0	51.1	7.19	7.09
E 86	21	37.95	64.3	54.6	7.45	7.37
E 78	24	33.78	65.0	58.4	7.82	7.47
E 98	24	39.11	65.3	52.9	7.99	6.66
Average	13	39.15	63.0	53.2	7.51	7.09

Tables 10, 11, and 12 give the mean composition of the bones of dairy cattle six months to two years of age fed at this Station on normal rations containing moderate amounts of Ca and P. The mineralization of the skeleton, altho not optimum, is to be regarded as representative of the average condition of normal dairy animals reared in the United States. Tables 13, 14, and 15 give similar results for dairy cattle suffering from typical phosphorus deficiency as it is frequently encountered in the Minnesota phosphorus-deficient area. The deficient condition in each case was produced experimentally at this Station. The mean composition of the bones shown for each animal represents the average result obtained on analysis of a longitudinal section of the right femur and humerus and of the entire sixth and eleventh ribs from the right side. The methods of analysis are described elsewhere in this bulletin.

Table 13

Mean Composition of Fresh Bones of Phosphorus-Deficient Dairy Cattle

Animal	Age	Ash	Alcohol-ether extract	Residue*	H_2O
	months	per cent	per cent	per cent	per cent
E 141	11	27.0	26.1	19.1	27.8
E 147	20	25.0	23.1	18.3	33.6
E 148	23	26.4	25.5	18.9	29.2
E 123	24†	27.0	26.1	19.1	27.8
E 121	28†	29.6	27.6	19.4	23.4
E 122	32†	29.9	26.6	19.6	23.9
E 73	50†	34.8	33.7	21.4	11.1
Average		28.6	27.0	19.4	25.3

* 100 — (ash + alcohol-ether extract + water.)

† Approximate age.

Table 14

Mean Composition of Dry Bones of Phosphorus-Deficient Dairy Cattle

Animal	Age	Ash	Alcohol-ether extract	Residue*
	months	per cent	per cent	per cent
E 141	11	36.0	34.7	29.3
E 147	20	37.4	34.9	27.7
E 148	23	36.9	36.0	27.1
E 123	24†	37.6	35.5	26.9
E 121	28†	38.6	35.8	25.6
E 122	32†	39.3	34.6	26.1
E 73	50†	39.1	37.8	23.1
Average		37.8	35.6	26.5

* 100 — (ash + alcohol-ether extract).

† Approximate age.

Table 15
Mean Composition of Dry Extracted Bones of Phosphorus-Deficient Dairy Cattle

Animal	Age	Organic residue and minor minerals*	Total ash	$Ca_3(PO_4)_2$	$CaCO_3$	$\frac{Ca_3(PO_4)_2}{CaCO_3}$
	months	per cent	per cent	per cent	per cent	
E 141..........	11	43.99	55.6	47.8	8.21	5.82
E 147..........	20	43.30	57.3	47.7	9.00	5.30
E 148..........	23	42.41	57.6	49.0	8.59	5.73
E 123..........	24†	40.58	58.7	49.9	9.52	5.24
E 121..........	28†	38.12	63.3	52.4	9.48	5.55
E 122..........	32†	39.20	60.4	52.1	8.70	5.99
E 73..........	50†	38.71	62.8	52.1	9.19	5.66
Average........		40.90	59.4	50.1	8.95	5.61

* 100 — $[Ca_3(PO_4)_2 + CaCO_3]$.
† Approximate age.

A brief description of each animal is given below.

Normal Animals

E 136 Grade Holstein bull calf. Fed whole milk for first few weeks, then corn, oats, alfalfa hay, and skimmilk until slaughtered.

E 137 Same as E 136. Blood plasma phosphate before slaughter 7.46 mgm. per 100 cc.

E 132 Scrub-bred bull calf. Fed practically the same as E 136 and E 137 except that the roughage fed consisted of equal parts of timothy and alfalfa hay. Blood plasma phosphate before slaughter 6.90 mgm. per 100 cc.

E 114 Grade Jersey bull calf. Fed very much like the previously mentioned animals. Some bran was fed with the grain and the roughage was mostly alfalfa hay with some timothy. Blood plasma phosphate ranged from 5.43 to 9.17 mgm. per 100 cc. at bimonthly tests made during a six months period before slaughter.

B 163 Purebred Jersey bull calf. The animal was considered normal for its age. He had never been fed deficient rations.

E 86 Grade Jersey heifer. Fed normally throughout its life. Was subject to indigestion and periods of losing weight but made rapid gains during the last four months before slaughter, and was almost 100 per cent normal in weight at that time. Blood plasma phosphate averaged 7.50 mgm. per 100 cc. for a year before slaughter.

E 78 Grade Holstein heifer. Fed the same as the purebred animals in the University herd including pasture in summer and grain mixture, alfalfa hay, and corn silage in winter. Animal was a good representative of her breed. Blood plasma phosphate averaged 6.00 mgm. per 100 cc. for a year before slaughter.

E 98 Grade Holstein heifer. Reared on normal rations and later fed corn, oats, linseed oilmeal, alfalfa and timothy hay. The animal made normal gains and was an excellent example of a normal heifer. Blood plasma phosphate averaged 6.84 mgm. per 100 cc. for 18 months before slaughter.

Phosphorus-Deficient Animals

E 141 Grade Holstein bull calf. Placed on phosphorus-deficient ration and skimmilk at age of four and one-half months. Was on full phosphorus deficient ration of beet pulp, molasses, low-phosphorus prairie hay, some alfalfa hay, and low-phosphorus grain at age of six months. Remained on this ration for five months, until slaughtered. Developed marked pica, lost weight after a time, showed increasingly unthrifty appearance and moved about as if in pain. Blood plasma phosphate averaged 3.25 mgm. per 100 cc. for three and one-half months before slaughter.

E 147 Grade Holstein heifer. Placed on phosphorus-deficient ration when about six months of age. This was continued for three months, when phosphorus intake was increased for a period of two months. Low-phosphorus ration was then resumed and continued for one year, until slaughtered. Blood plasma phosphate concentration declined and remained at approximately 2.5 mgm. per 100 cc. Animal exhibited symptoms characteristic of phosphorus deficiency and became very stiff and nearly helpless just prior to slaughter.

E 148 Grade Holstein heifer. Placed on phosphorus-deficient ration at six months of age. Ration continued for two months, then a more liberal supply of phosphorus was given. Low-phosphorus ration resumed and continued for one year, until slaughtered. The effects on blood phosphate, and on the appearance and general condition of the animal were practically identical with those observed for E 147.

E 123 Purebred Holstein heifer. Purchased when about seven months of age. Placed on phosphorus-deficient ration when nine months of age and continued on this ration for 14 months. First symptoms were noted after seven to eight months and the animal showed all the characteristics of the disease for at least six months before slaughter.

E 121 Grade Holstein heifer. Purchased when about 11 months of age. When about 13 months old was placed on phosphorus deficient ration. Symptoms began to appear after a further reduction in phosphorus intake and for the last few months before slaughter. After having been on the ration for 15 months, marked stiffness and general inertia developed. Pica disappeared during this time, but the general physical condition became worse. Blood phosphate remained at a low level, averaging 2.04 mgm. per cent of the plasma for seven months prior to slaughter.

E 122 Grade Holstein heifer. Purchased when about 15 months of age. Placed on phosphorus-deficient ration at 17 months of age and continued on this ration for about 15 months, when she was slaughtered. She never showed extreme symptoms of phosphorus deficiency. Plasma phosphate never fell below 3.33 mgm. per cent.

E 73 Grade Shorthorn cow. Purchased when about three years of age. Was thin, somewhat undersized, and was milking but not bred. Fed a phosphorus-deficient ration for about 17 months and then slaughtered. Monthly blood analyses for seven months prior to slaughter averaged 2.1 mgm. per cent inorganic phosphate. Phosphorus deficiency symptoms were marked during this period.

Tables 10 and 13 give the composition of the fresh bones, scraped free from adhering flesh. Tables 11 and 14 give the composition of dried bones, and Tables 12 and 15 of the dry alcohol-ether extracted bones. Figure 2 shows a diagrammatic comparison of the average composition of the bones of all the animals in each group. The tables and the diagrams, especially, show very clearly the principal characteristics of the skeletal condition from a chemical standpoint as it is affected by phosphorus deficiency.

In the fresh bone the primary effect of phosphorus deficiency is the marked reduction in the ash and its replacement by lipid material. The same effect, altho to a less extensive degree, was obtained by Henderson and Weakley (26). The bones in our phosphorus-deficient animals contained 77.8 per cent of the ash of the normal animals and 153 per cent of the lipids in contrast to 90 per cent ash and 108 per cent lipids as found by Henderson and Weakley (see Table 9). A marked increase in water does not occur in the samples analyzed by us, Henderson and Weakley finding a 17 per cent increase in water in the bones in phosphorus deficiency. The water content of the normal and phosphorus-deficient bones is essentially the same in our experiments, altho there is a slightly higher water content in the phosphorus-deficient bones.

The low ash and high lipid content of the fresh bones is greatly magnified when the analyses are compared on the dry basis, altho the percentage change is the same.

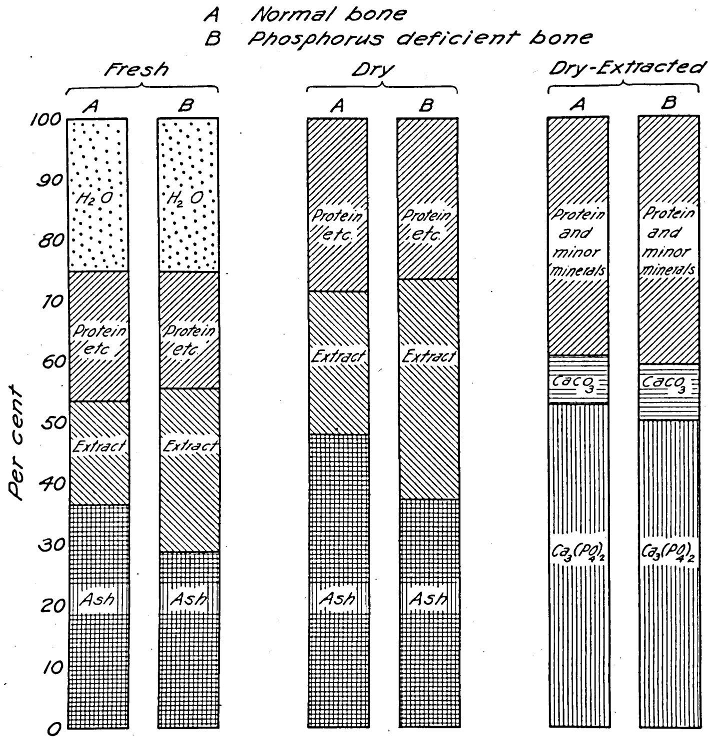


Fig. 2. Diagrammatic Distribution of Bone Constituents in Bone of Normal and Phosphorus-Deficient Dairy Cattle

The characteristic feature of the comparison on the dry-extracted basis is the low total ash and the lower $Ca_3(PO_4)_2$ and the higher $CaCO_3$ content of the bones from the phosphorus-deficient animals. The changes in the relative proportion of the principal mineral salts produce the same effect on the ratio of $Ca_3(PO_4)_2$ to $CaCO_3$ as was found in the preliminary studies (7). The average ratio of these compounds found in the bones of the normal animals here reported is 7.08, which corresponds almost exactly with the value 7.02 found for 20 animals, previously published (7). The average value of the $Ca_3(PO_4)_2$:$CaCO_3$ ratio of the bones of the seven phosphorus-deficient animals reported in this bulletin is 5.61. The average for four animals previously reported is 5.30. One animal is included in both averages. The preliminary report is thus completely confirmed. The effect of phosphorus deficiency on the $Ca_3(PO_4)_2$:$CaCO_3$ ratio of the bones of cattle is clearly a specific effect, characteristic of the deficiency.

INORGANIC PHOSPHORUS SUPPLEMENTS VERSUS PHOSPHORUS IN FEEDS IN RELIEVING PHOSPHORUS DEFICIENCY

Some difference of opinion has been expressed by nutrition workers regarding the relative utilization of natural and inorganic mineral supplement forms of phosphorus by animals. On the other hand, the ability of animals to survive on rations containing only inorganic phosphorus has never been demonstrated. It is, however, recognized that ortho phosphoric acid ingested in different forms is taken up by the body in both organic and inorganic forms. Our work, as well as that of Theiler (13,18) and others, has also demonstrated that inorganic phosphates such as bonemeal, tricalcium phosphate, and sodium phosphate are utilized by cattle when fed in conjunction with phosphorus-deficient rations.

The significance of the question to livestock owners in regions where phosphorus-deficiency troubles occur in cattle is apparent, for on the answer depends the method to be followed in correcting and preventing the condition.

The experiment here reported was designed to compare the efficiency of phosphorus provided in a readily soluble inorganic form with that of the forms of this element as found in natural foodstuffs. The results were measured by changes in the inorganic phosphorus content in the blood plasma of the experimental animals and by observations as to the physical symptoms of phosphorus deficiency which were exhibited by all the animals when the experiment was begun. This method of measuring the results was selected because our previous studies had shown that the inorganic phosphorus content of the blood in phosphorus-deficient animals responds very quickly to changes in the quantity of phosphorus absorbed.

The experiment consisted of two trials. A record of the rations fed, the nutrient and mineral intake for the animals in both trials is found in Table 16. These rations provided approximately the same percentage of the nutrient requirements of the animals according to the Morrison standard. In the first trial the following three animals were used:

E 118, grade Holstein heifer, about 3 years old, weight 1,134 pounds.

E 125, grade Holstein heifer, about 2 years old, weight 707 pounds.

E 120, grade Holstein heifer, about 2½ years old, weight 1,003 pounds.

Table 16

Feed Intake

Natural Foodstuffs vs. Inorganic Phosphorus Supplement

No. of animal	Length of period	Average of weights during period	Daily ration: Prairie hay	Daily ration: Grain mixture	Daily ration: Molasses	Daily ration: $NaH_2PO_4 \cdot H_2O$	Nutrients in ration: Crude protein	Nutrients in ration: Total digestible nutrients	Mineral intake, per day: Ca	Mineral intake, per day: P
	days	lb.	lb.	lb.	lb.	gm.	lb.	lb.	gm.	gm.
E 118	16	1,136.5	14.69	3.00	2.00	...	1.02	9.85	50.15	14.55
	80	1,194.6	15.29	3.91	2.00	...	1.21	10.82	52.23	17.58
	73	1,299.4	16.59	4.00	2.00	...	1.22	11.14	55.96	24.01
E 125	16	708.3	10.28	3.00	2.00	...	0.90	7.92	38.10	13.16
	80	767.6	11.29	3.96	2.00	...	1.11	9.10	41.29	16.47
	73	875.9	12.26	4.00	2.00	...	1.10	9.24	44.11	22.64
E 151	147	762.6	12.86	3.00	2.00	...	0.94	9.14	39.87	13.62
E 152	84	458.6	9.10	3.00	2.00	...	0.85	7.34	31.13	12.61
E 120	16	1,005.0	14.00	3.00	2.00	20.0	1.12	9.54	47.35	12.57
	37	1.037.5	13.77	4.00	2.00	28.0	1.35	10.20	46.89	15.32
E 150	147	696.7	11.3	3.00	2.00	21.0	1.07	8.51	35.38	11.71
E 153	84	790.6	12.8	3.00	2.00	21.0	1.10	9.04	38.91	11.99

These animals had been on a low-phosphorus ration for more than a year previous to the time the trial was started. The inorganic phosphorus in their blood plasma was therefore low, about 2.00 mgm. per 100 cc. and had been at this point during the previous six months. The rations of E 118 and E 125 were adjusted so as to provide an adequate but not an excessive supply of phosphorus in the form of foodstuffs. E 120, the other heifer, was provided with a ration of natural food stuffs of low phosphorus content to which was added an amount of sodium phosphate sufficient to bring her phosphorus intake to a level approximately that of the other two.

The animals were started on experiment on November 21, 1929, but after continuing for 16 days with little or no response in the inorganic phosphorus content of the blood plasma, it became apparent that the intake of phosphorus in all the rations was inadequate and consequently an increase of approximately 20 per cent was made in each. This resulted in an immediate increase in the inorganic phosphorus in the blood plasma of E 120, as can be observed in Table 17. A noticeable response was also shown in the blood of the other two animals, but it was much less abrupt and the rise was not nearly so great. Owing to the fact that E 120 gave a positive reaction to the tuberculin test she was discontinued on experiment on January 12, 1930, making further comparison impossible. The two receiving natural foodstuffs supplement were continued on experiment, but it soon became apparent that, altho they showed improvement in general appearance and activity, the inorganic phosphorus in the blood plasma did not approach what may be considered normal for cattle at that age. This is distinctly apparent from the data in Table 17. Furthermore, the blood phosphate showed a tendency to drop after several months on the ration. At this point it therefore was decided to increase again the natural forms of phosphorus in the ration fed. When this was done the inorganic phosphorus in the blood plasma showed an immediate response, rising to a point well about the normal where it remained during the rest of the trial.

The second trial was started on June 20, 1930, and was conducted in the same manner as the first. Four cows of mixed or scrub breeding were used. They were purchased for the purpose and came from farms in the phosphorus-deficient region in the state and all showed many of the characteristic symptoms of phosphorus deficiency, including depraved appetite and low inorganic phosphorus in the blood plasma. Three of the animals, E 150, E 151, and E 153, were mature cows, open or only recently bred. The other, E 152, was an open heifer about 3½ years old but much undersized. The weight of each animal on the date the experiment started was as follows:

E 150, 609 pounds; E 151, 720 pounds; E 152, 404 pounds; E 153, 743 pounds.

Two of the cows, E 151 and E 152, were assigned rations supplying their needed phosphorus in natural forms. The other two, E 150 and E 153, received a ration low in natural forms of phosphorus but supplemented by a sufficient amount of inorganic phosphorus as sodium phosphate ($NaH_2PO_4 \cdot H_2O$) to bring the total intake of this element up to that fed the cows on the other ration.

It is extremely difficult to decide on a definite basis of phosphorus feeding under conditions in which wide differences in weight exist, as in the present trials. The question is, shall the individual animal, regardless of its size, be considered as the unit or should the phosphorus intake be determined on the unit weight (1,000 pounds) basis? In the present trial both factors were given consideration as was also the general physical condition of each animal. Our experience in the first trial indicated the approximate intake necessary to obtain results, consequently no changes were found necessary during the second trial. From the responses shown in the inorganic phosphorus in the blood plasma of the animals when placed on experiment it is evident that the phosphorus intake was sufficiently high in each case. It is noteworthy in this connection that there is no distinct difference between the pair given inorganic phosphorus and that given natural foodstuffs in the promptness and degree of response shown. In this respect these groups differ quite distinctly from those in the first trial. One animal from each pair, E 152 and E 153, respectively, was discontinued after being on experiment 84 days. The other two, E 150 and E 151, completed 147 days on the experiment.

Prairie hay, obtained from low-phosphorus soil, was the only roughage fed during both trials. During the entire time they were on experiment E 151 and E 152 were fed a grain mixture composed of three parts each of ground corn and cottonseed meal and one part of ground oats.

This mixture, also, was fed to E 118 and E 125 during the period November 21, 1929, to February 24, 1930, inclusive. During the rest of their experimental periods these two animals received a mixture of two parts each of cottonseed meal and wheat bran, and one part each of ground corn and ground oats.

E 120, E 150, and E 153, during their respective experimental periods, were fed a grain mixture composed of four parts corn gluten meal, two parts ground corn, and one part ground oats. The animals

Table 17

Blood Analyses

Natural Foodstuffs vs. Inorganic Phosphorus Supplement. Total Calcium and Inorganic Phosphorus in 100 cc. of Blood Plasma

Date	Natural foodstuffs supplement				Inorganic phosphorus supplement	
	E 118		E 125		E 120	
	Ca	P	Ca	P	Ca	P
1929	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Nov. 20	11.62	2.57	11.49	2.29	10.93	3.18
" 21	10.73	2.71	11.43	2.41	10.86	2.96
" 22	11.68	2.95	11.49	2.66	10.99	4.29
" 23	11.30	3.20	11.24	2.50	10.55	3.13
" 25	11.18	2.44	11.56	2.24	10.86	3.65
" 27	11.24	3.08	10.48	1.98	10.55	3.62
" 29	11.24	2.93	11.49	2.15	10.55	3.41
Dec. 2	11.30	3.54	10.74	3.18	10.61	3.75
" 4	10.67	3.26	10.42	3.26	10.36	4.25
" 6	10.67	3.13	11.12	2.45	9.35	3.85
" 9*	9.85	2.83	10.86	2.65	9.48	4.12
" 11	10.36	3.38	10.74	2.64	9.92	4.45
" 13	9.85	3.03	9.48	2.88	9.72	5.81
" 16	10.10	3.41	10.55	2.78	9.72	5.52
" 19	11.11	3.05	10.80	3.88	10.04	5.33
" 21	9.98	3.00	10.42	3.33	10.10	5.44
" 23	10.93	3.29	10.63	3.41	9.73	5.99
" 26	10.80	3.78	11.56	3.51	9.41	6.12
" 28	10.48	3.38	11.31	3.04	9.60	6.45
" 31	10.17	4.07	10.86	3.11	....	....
1930						
Jan. 2	10.48	4.10	10.99	3.99	....	....
" 4	10.99	4.33	10.73	2.90	....	....
" 6	10.67	4.21	11.18	3.49	....	....
" 8	10.55	3.61	10.93	2.81	9.75	5.65
" 10	10.48	3.95	10.92	3.65	....	....
" 13	11.50	3.81	11.11	3.07	....	....
" 15	10.67	4.04	10.74	4.14	....	....
" 17	11.18	3.87	11.05	3.59	....	....
" 20	10.67	4.26	10.67	3.77	....	....
" 22	10.90	3.81	11.11	3.17	....	....
" 24	11.05	3.68	10.86	3.26	....	....
" 27	10.87	3.20	10.90	4.29	....	....
" 29	10.73	3.68	10.96	3.14	....	....
" 31	10.90	2.77	10.67	2.43	....	....
Feb. 3, 5, 7	10.90	3.51	11.07	2.77	....	....
" 10, 12, 14	10.90	3.08	11.30	2.81	....	....
" 17, 19, 21	11.01	3.37	11.19	2.48	....	....
" 24	11.01	3.99	10.96	2.60	....	....
" 26†	10.96	3.71	10.78	2.78	....	....
" 28	10.61	4.58	10.90	3.27	....	....
Mar. 3	10.44	4.85	10.15	5.01	....	....
" 5	10.27	4.62	11.53	4.29	....	....
" 7	10.38	4.13	11.13	3.88	....	....
" 10	9.81	6.71	10.79	4.38	....	....
" 12	8.60	7.26	9.64	5.87	....	....
" 14	10.44	7.33	10.84	6.60	....	....
" 17, 19, 21	10.96	5.94	11.93	5.62	....	....
" 24, 26, 28	9.52	6.29	10.55	6.10	....	....
Apr. 30, May 1, 2	10.97	4.78	11.15	5.77	....	....

* Phosphorus intake increased about 25 per cent.

† Phosphorus intake increased about 35 per cent.

in both trials also received 2 pounds of black strap molasses daily. Salt was provided *ad libitum.* All feedstuffs were sampled and analyzed. Data giving the chemical composition of the various feedstuffs and the periods when they were fed are presented in Appendix tables 13 and 14.

Blood samples were collected for chemical analysis at frequent intervals, usually each morning. Complete data are presented in Table 18.

Table 18

Blood Analyses

Natural Foodstuffs vs. Inorganic Phosphorus Supplement. Total Calcium and Inorganic Phosphorus in 100 cc. of Blood Plasma

Date	Inorganic phosphorus supplement				Natural foodstuffs phosphorus supplement			
	E 150		E 153		E 151		E 152	
	Ca	P	Ca	P	Ca	P	Ca	P
1930	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
June 4-6	11.35	2.67	9.22	2.45	11.52	3.14	10.82	2.75
" 17	...	2.21	...	2.49	...	2.72	...	2.40
" 18	...	2.32	...	2.67	...	2.82	...	2.80
" 19	...	2.00	...	2.45	...	2.10	...	2.40
" 20*	...	1.64	...	2.20	...	2.56	...	2.32
" 21	...	1.84	...	1.93	...	2.43	...	2.64
" 23	...	3.27	...	3.33	...	2.37	...	3.25
" 25	...	2.70	...	2.88	...	3.85	...	3.48
" 27	...	4.78	...	2.70	...	4.43	...	3.65
" 30	...	4.77	...	3.26	...	4.46	...	4.02
July 2	...	4.95	...	4.37	...	4.52	...	3.57
" 5	9.76	4.92	8.63	4.61	8.80	4.96	10.67	4.58
" 7	...	5.32	...	4.69	...	6.10	...	3.77
" 9	...	5.32	...	4.45	...	7.19	...	4.50
" 11	9.14	5.35	8.63	4.17	9.54	5.40	10.90	3.61
" 14	...	5.34	...	4.52	...	4.24	...	4.42
" 16	8.46	6.45	9.03	4.47	10.16	5.73	10.56	4.13
" 18	...	6.12	...	5.41	...	5.62	...	3.29
" 24	9.93	5.92	9.31	6.76	9.99	4.98	10.84	8.00
" 26	...	6.13	...	6.37	...	6.13	...	7.81
" 28	...	6.67	...	7.75	9.76	6.06	...	7.41
" 30	10.22	4.59	9.31	6.92	...	5.65	9.76	5.65
Aug. 1	...	7.22	...	7.04	...	6.37	...	6.37
" 4	...	5.75	...	5.13	...	5.81	...	6.29
" 6	10.45	4.39	10.20	4.48	10.82	4.31	11.57	5.37
" 8	...	5.41	...	5.51	...	6.03	...	6.51
" 11	...	5.00	...	4.40	...	6.41	...	6.63
" 14	...	5.43	...	4.52	...	5.82	...	5.95
Sept. 2, 3, 4	11.38	6.62	11.61	5.02	11.38	6.60	11.95	5.88
" 22, 23, 24	10.81	7.12	...	...	10.95	5.01	...	...
Oct. 2, 3, 4	10.24	6.68	...	...	11.15	7.14	...	...
Nov. 3, 4, 5	11.25	6.02	...	...	12.06	5.43	...	...

* Beginning of experimental period.

The animals were weighed once every ten days and on three successive days every thirty days. They were turned out in a dry lot for exercise every day except during extremely cold or stormy weather.

From a consideration of the data presented in Tables 16 and 17, indicating the response shown in the inorganic phosphorus in the blood plasma of the various animals, it becomes apparent that inorganic phosphorus supplement as well as organic phosphorus in foodstuffs alike may be used successfully in supplementing a low-phosphorus ration for cattle. If there is any difference in efficiency between the two, as indicated by the results, it is in favor of the inorganic form. Altho the results are complicated by the fact that animals of different ages and sizes were used in the trials, yet it appears that a slightly higher intake of phosphorus as foodstuffs than in the inorganic form employed was necessary to obtain equal results. This is shown clearly in the first trial in both the promptness and the degree of response in the inorganic phosphorus in the blood plasma following the first increase in the phosphorus intake on December 7, 1929.

While no such difference is apparent in the blood of the two groups during the second trial, owing, perhaps, to the fact that these animals were provided with a somewhat higher phosphorus intake from the start, yet a similar difference in the rate of recovery is indicated by the more rapid rate of gains on the part of the animals receiving the inorganic phosphorus supplement. The differences indicated in the economy of gains between the two groups in both trials may also be of some significance. These facts are clearly indicated in Table 19.

Table 19

Natural Foodstuffs vs. Inorganic Phosphorus Supplement in Relation to Gain in Weight

No. of animal and form of phosphorus	Average daily gain	Minerals in ration per 1,000 lb. live weight		Total digestible nutrients received above maintenance	
		Ca	P	Per day	Per lb. gain
	lb.	gm.	gm.	lb.	lb.
First trial					
E 118 Natural	0.90	43.07	18.48	1.70	1.89
E 125 Natural	1.42	50.36	25.85	1.57	1.11
E 120 Inorganic	1.65	45.20	14.77	2.08	1.26
Second trial					
E 151 Natural	0.63	52.28	17.86	1.92	3.05
E 152 Natural	0.93	67.88	27.50	2.26	2.43
E 150 Inorganic	1.14	50.77	16.80	1.64	1.44
E 153 Inorganic	1.39	49.22	15.17	1.69	1.22

The results in the first period of E 120 and from the first two periods of E 118 and E 125 during the first trial have little significance, as the amount of phosphorus provided in the rations and supplements was evidently too low. For this reason they are not given in the table.

In arriving at the results presented in the last two columns in Table 19 the maintenance requirements determined by Gullickson and Eckles (33) were used for animals weighing 1,000 pounds or less. For weights beyond this the requirements were calculated according to the following formula, using 7.925 pounds of total digestible nutrients at 1,000 pounds:

$$\text{Maintenance} = 7.925 \left(\frac{\text{Desired weight}}{1{,}000}\right)^{2/3}$$

From the results presented, it is apparent that the livestock growers in regions of phosphorus-poor soil are wholly justified in their present practice of feeding their cattle mineral feeds rich in phosphorus. This statement, however, does not take into consideration the considerable benefits that usually accrue from the other nutrients, particularly protein, contained in the feedstuffs rich in natural forms of phosphorus. Also our experiments do not show specifically that all mineral phosphates will have exactly the same value per unit of phosphorus as $NaH_2PO_4 \cdot H_2O$ in restoring phosphorus-deficient animals to normal condition. They should not be interpreted as a blanket recommendation of all forms of mineral phosphate that may be offered for sale in phosphorus-deficient regions.

RELATION OF PHOSPHORUS CONTENT OF THE SOIL TO THAT OF FORAGE GROWN ON IT AND TO PHOSPHORUS DEFICIENCY IN CATTLE

Mineral analyses of feedstuffs grown on farms on which cattle show the characteristic symptoms previously described point definitely to an extremely low phosphorus content in the roughage fed as the chief causative factor. This condition is, as has been suggested by Eckles, Becker, and Palmer (1), clearly due to the phosphorus-poor state of the soil on which the forage crops are grown. This is indicated by the fact that the trouble is encountered only in regions of the state where, according to Alway (34), the soil is known to be phosphorus-poor. The direct relationship between the mineral content of the soil and the amount of minerals in the plants grown on it has also been pointed out by many others, including Alway (34) and Nygard (35). Orr (36), in a report prepared under his supervision, gives a comprehensive review of the literature dealing with this subject, including citations.

The following figures give the average calcium and phosphorus content of hay grown on the low-phosphorus soil in Minnesota where phosphorus deficiency occurs among cattle compared with analyses from

Michigan (37) and average analyses as given by Henry and Morrison (38):

	No. of samples	Ca per cent	P per cent	Mg. per cent
Prairie hay				
Survey of low P area in Minnesota...	51	0.440	0.106	0.233
Used in experimental work...........	21	0.426	0.071	0.270
Seven years from Nygaard farm......	24	0.410	0.064	0.258
Timothy hay				
Survey of low P area in Minnesota...	5	0.395	0.112	0.197
Minnesota market	12	0.319	0.144	0.151
Acid soil	11	0.288	0.141	0.124
Henry and Morrison average..........	..	0.179	0.135	0.102
Michigan, average of 6 years..........	..	0.332	0.134	....
Alfalfa hay				
Survey of low P area in Minnesota...	24	1.810	0.212	0.397
Minnesota market	22	1.390	0.210	0.314
Michigan, average of 6 years..........	..	1.510	0.177	....
Henry and Morrison average	..	1.390	0.235	0.456

Detailed analyses of the above samples are found in Appendix tables 15 to 20. The extremely low phosphorus content of the prairie hay is evident. Analyses are not available of prairie hay grown on other types of soil. Presumably, it would be somewhat higher but the probabilities are that hay of this type will be relatively low under any condition. Such comparisons would have a limited value at best, because hay of this kind is a composite of a variety of species native to the region and the kind and proportion of each will undoubtedly depend upon the soil and environmental conditions under which it is grown. Even in the same field a decided variation is possible, especially between upland and lowland where the soil has a peaty character. Two samples of prairie hay purchased on the general market showed practically the same phosphorus content as that from the low-phosphate soil area. The origin of this market hay is not known, but probably it, also, came from the low-phosphorus region.

Alfalfa hay shows a significantly higher phosphorus content than prairie hay when grown on the same type of soil. It is probable, however, that the small yield of alfalfa on soils decidedly deficient in phosphorus results in a selection of crops by the farmers and as a result the alfalfa samples did not represent farms where the soil is the most lacking in phosphorus. The higher phosphorus content of alfalfa explains the observation that little phosphorus-deficiency trouble is experienced when this hay is fed. For seven years prairie hay for experimental purposes has been purchased from a particular farm described in this report as the Nygaard farm. Table 20 shows the composition of this hay for seven consecutive years.

Table 20

Mineral Content of Prairie Hay Grown on the Nygaard Farm Over a Period of Seven Years

Year	No. of samples	Dry matter	Ash	Ca	P	Mg
		per cent	per cent	per cent	per cent	per cent
1925	6	92.0	6.59	0.404	0.094	....
1926	2	92.3	....	0.316	0.047	....
1927	3	85.3	7.15	0.289	0.067	....
1928	4	86.6	5.86	0.420	0.068	0.186
1929	1	88.7	6.44	0.494	0.057	0.235
1930	5	83.7	5.92	0.475	0.055	0.307
1931	3	86.0	7.75	0.470	0.059	0.300
Av. of 7 years	..	87.8	6.61	0.410	0.064	0.258

In our first publication, attention was called to the fact that phosphorus-deficiency troubles, as found in Minnesota, appear early in the fall and winter and are more severe during a winter following a summer of subnormal rainfall. Further experience has verified this conclusion. The same observations have been reported by practically all who have investigated mineral-deficiency troubles, including Tuff (39).

There appear to be several reasons for the pronounced effect of subnormal rainfall upon the severity of phosphorus deficiency in cattle. First of all, during a dry summer the pasture grass is scant and is limited chiefly to the lowland, where the mineral content of the soil is lower than on the higher levels. As a result of this condition the animals consume grass especially low in phosphorus during the season when they normally replenish their supply by storage in the bones. During a very dry season the symptoms of phosphorus deficiency shown by cattle at the beginning of the pasturing season may not disappear during the grazing season as is common during a year of normal pasturage, but may actually become more pronounced. Furthermore, the herbage which grows even on the higher soil has a somewhat lower phosphorus content in a dry year than in one of normal rainfall. As a result of this combination of conditions the effect of a dry summer is to make more serious the phosphorus deficiency the following winter. It is also probable that less important factors, such as the stage of cutting and the method of curing, have some influence upon the phosphorus content.

The variation in phosphorus content of hay from the same field as a result of the interaction of various factors is shown by Table 20, in which is given the mineral composition of hay from the Nygaard farm for seven seasons. Note that for 1926 the phosphorus content was exactly half that for 1925. It is interesting to note, also, that at no time during the seven years did the average composition in any year show more than 0.1 per cent phosphorus. Table 21 gives the mineral content of samples of alfalfa, timothy, and prairie hay taken on successive years when the rainfall varied about 20 per cent. These data only in part represent the same fields but include all samples taken during the years in the discussion.

Table 21

Mineral Content of Hays from Low-Phosphorus Soil as Affected by Rainfall

Variety	Annual rainfall	No. of samples	Ca	P	Mg
	in.		per cent	per cent	per cent
Alfalfa hay	17.49	4	1.903	0.132	0.469
Alfalfa hay	21.98	10	1.891	0.212	0.411
Timothy hay	17.49	2	0.465	0.096	0.238
Timothy hay	21.98	2	0.322	0.118	0.151
Prairie hay	17.49	12	0.379	0.067	0.188
Prairie hay	21.98	18	0.437	0.123	0.231

A calculation of the estimated phosphorus requirement of an average lactating cow and the amount supplied by the typical ration she is likely to receive in the phosphorus-deficient area in this state shows clearly why trouble results. According to Armsby (40), basing his statement upon the work of Henneberg and Stohmann, and Kellner, a 1,000-pound cow producing 20 pounds of milk daily would require 10 grams of phosphorus for maintenance and 15 grams for milk production, a total of 25.0 grams. In the phosphorus-deficient area the cow probably would be fed about 22 pounds of prairie hay, and 6 pounds of oats daily. Using the seven-year average for the Nygaard farm, 0.064 per cent of phosphorus in the prairie hay would give 6.39 grams from this source. Average figures for oats would give an approximate intake of 11.89 grams from the grain, a total of 18.28. With this ration the intake would be 7.85 grams, or 31.4 per cent, below the estimated requirement. It is true that the ration suggested would not be considered by experts as suitable for high-class dairy production, but it is rather above the average actually used in the districts studied. Prairie hay, however, is a better feed than is often realized when the phosphorus deficiency is supplied by a suitable supplement. Evidence of this is seen by the fact that cow E 33, as reported in Table 23, produced 8,658 pounds of milk in a year on a ration of prairie hay and oats supplemented with sodium phosphate.

RELATION OF PHOSPHORUS DEFICIENCY TO FEED REQUIREMENTS FOR PRODUCING MILK AND GAIN IN LIVE WEIGHT AND TO THE UTILIZATION OF FEED

Among the symptoms of phosphorus deficiency in cattle, as it occurs on farms, is a general unthrifty, run-down condition of the animals and a lack of appetite for roughage. Cattle owners complain that their animals remain in thin flesh and in an unthrifty condition regardless of what the owners consider liberal feeding. When such statements first were received in correspondence, we did not take them seriously because ideas of good feeding are flexible. However, a survey of over a hundred farms, as well as our experience in conducting experimental work on this project during several years, confirms this complaint. Cattle on farms where the trouble occurred, which was later found to be phosphorus deficiency, were clearly in a physical condition much below that of animals in areas close by where mineral deficiency symptoms never have been observed. Animals raised to maturity in this low-phosphorus area are usually undersized at maturity.

Our first experiments in feeding a phosphorus supplement (1) showed that astonishing results in the condition of the animal follow the addition of a phosphorus supplement to a ration lacking in this mineral. For example, E 58, received from the phosphorus-deficient area in a very bad condition and weighing only 498 pounds, weighed 860 pounds after a year of feeding a phosphate supplement with the same ration used in the deficient area. During this period she was milking ten months.

These results from E 58 are cited as typical of our experience. This animal, with others, is illustrated in Minnesota Bulletin 229 (1). The striking effect upon the amount of milk produced when phosphorus is added to a deficient ration is presented in another section of this report.

Observations regarding the effect upon gains in live weight of making up a phosphorus deficiency by the use of suitable supplements have also been reported by others, especially Theiler and associates (13). They found that the addition of bonemeal to the ration of a lot of 50 experimental cattle from a mixed herd resulted in an average gain of 105 pounds per head during the pasturing season in excess of the gain by a check lot not receiving the supplement. The controls gained 14 per cent in weight and the bonemeal lot 29 per cent. A second lot of young animals receiving bonemeal gained 42 per cent compared to 21 per cent for the controls. In these experiments by Theiler the animals were all grazed where the vegetation is low in phosphorus.

No feed records are available for the animals used in these experiments reported by Theiler. At the Minnesota Experiment Station complete feed records with chemical analyses of the components are available for many cattle receiving phosphorus-deficient rations. In reviewing our results and those of Theiler and others, it is evident that the effects of phosphorus on gains in live weight are due both to a stimulation of the poor appetite that usually accompanies phosphorus deficiency and to an increase in the utilization of the feed consumed.

Relation to Gain in Weight

The striking results from making up a deficiency of phosphorus in a ration are shown by data from cows E 73 and E 74. These animals were obtained from a farm in the phosphorus-deficient area and were approximately the same age and weight at the beginning of the trial. The two were rather thin in flesh in the beginning and were not lactating. Both animals received oats and low-phosphorus prairie hay grown on phosphorus-deficient soil. This is the most common feed used in the locality from which they came. The amount of nutrients to be supplied was calculated by the use of the Morrison Feeding Standard (15). The requirement for maintenance was first calculated and to this was added an arbitrary amount in order that the animal might have a margin for use in making a gain in body weight.

It was planned that the total feed intake should be about 130 per cent of that required for maintenance. The nutrients consumed as later calculated were not exactly on the same level as contemplated for the two animals. Owing to the more rapid increase in the weight of E 74, receiving the phosphorus supplement, her margin of nutrients above estimated maintenance was somewhat below that of E 73. The results are shown in Figure 3 and in the following statement:

Results of Adding a Phosphorus Supplement to a Phosphorus-Deficient Ration

	E 73	E 74
Days	235	235
Gain per day, lb.	0.30	1.31
T.D.N.[5] for maintenance per day, lb.	6.26	6.84
T.D.N. received per day, lb.	8.38	8.29
T.D.N. available above maintenance per day, lb.	2.12	1.45
T.D.N. per pound gain, lb.	27.93	6.32
T.D.N. above maintenance, per pound gain, lb.	7.06	1.10
Ca intake per day, gm.	29.46	25.95
P intake per day, gm.	7.13	22.90

[5] T.N.D. is an abbreviation for "total digestible nutrients." Digestible crude protein + digestible carbohydrates + (fat × 2.25) = T. D. N.

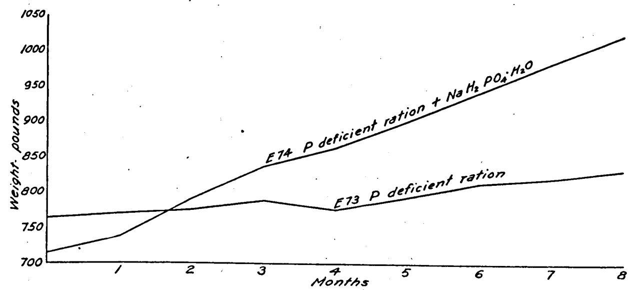


Fig. 3. Effect Upon Gain in Weight of Adding a Phosphorus Supplement to a Phosphorus-Deficient Ration

E 74 and E 73, non-lactating cows, received the same phosphorus-deficient ration fed at the same level (136 per cent of maintenance) as calculated from Morrison's Feeding Standard. E 73 gained 71 pounds in 235 days compared to 308 pounds by E 74.

Note the difference in gain per day, the uniformity of nutrient intake, the marked difference in the total digestible nutrients required per pound of gain, the total digestible nutrients above maintenance required for each pound of gain, and the wide variation in the phosphorus intake. The cattle owner who views the problem from the economic standpoint will note that a pound of gain by E 74, the animal receiving the phosphorus supplement, required only 22.6 per cent of the digestible nutrients required for the same gain by E 73 with the low phosphorus intake. A nutrition specialist will also be interested in the wide variation in the amount of digestible nutrients available above maintenance used per pound of gain. These figures indicate that the low phosphorus intake in some way interfered with the full utilization of the nutrients consumed.

Relation to Economy of Milk Production

Significant data regarding the economic questions involved are supplied by three cows. These animals were used in a trial to determine the effect upon milk production of supplementing a low-phosphorus ration with sodium phosphate. The data treated from another point of view are found in a section of this report dealing with the relation of the phosphorus intake to milk production. These cows were purchased shortly before or shortly after parturition from farms where phosphorus deficiency regularly occurred and brought to the Experiment Station. The ration fed was that used on the farms from which these animals came. The amount fed was regulated by the Morrison Feeding Standard. Each cow received sufficient nutrients for maintenance plus the milk she was producing with a margin of about 20 per cent. By this plan it was made certain that the milk production was not limited by the intake of protein or total digestible nutrients. The experiment was not set up especially to supply data concerning the subject under consideration.

The cows subject to the low-phosphorus intake had a relatively short milking period. The objective of the experiment was to compare the production of the same cows continued through another lactation period on the same ration with the addition of a supplement to insure a sufficient supply of phosphorus. In order that the cows might have time to replenish their reserve, the feeding of a phosphorus supplement was begun as soon as the cows were dry, at the end of the lactation period on the low-phosphorus ration.

This procedure makes it difficult to compare the nutrients used for milk production during the lactation period on a low-phosphorus ration with those in the next period when this deficiency is corrected. Normally, a cow should freshen at about 12-month intervals. Her maintenance while dry is part of the expense of milk production, measured either in nutrients or money values. As explained, part of the effect of a low phosphorus intake is to shorten the lactation period. Clearly, from a practical standpoint, to measure the nutrient cost of milk per unit of milk produced, a 12-month period must be included. In the case of the cows on the low-phosphorus ration, the feeding of a phosphorus supplement was begun when they were dry; hence the nutrients received during this dry period could not be added to those received during the milking period. It seemed advisable to estimate the nutrients required for the maintenance of the cow from the time she was dry until the end of the 12-month period. An estimate based on the Morrison standard was therefore made of the nutrients required for maintenance by the cow from the time she was dry until the end of the 12-months period. It is well established that these maintenance figures closely represent the actual requirements of an animal.

The figures for total nutrients as used, therefore, represent the actual amounts received during the milking period, calculated by applying average digestion coefficients to the chemical analyses of the feedstuffs, plus an allowance for maintenance during a dry period necessary to complete a full 12 months.

The following figures show the results for three cows:

Effect of Phosphorus Intake on Economy of Milk Production (a) Phosphorus-Deficient Period, (b) Next Lactation Period, Same Character of Ration with Phosphorus Supplement

	Milk	Total digestible nutrients per pound of milk	Average daily phosphorus intake
	lb.	lb.	gm.
E 93			
(a)	3,543	1.13	14.2
(b)	8,745	0.72	43.0
E 75			
(a)	3,276	1.26	14.9
(b)	5,017	0.91	35.7
E 94			
(a)	2,913	1.20	12.7
(b)	5,015	0.87	39.2

These figures show in a striking manner the increased cost for feed of producing milk from a ration deficient in phosphorus. The three cows on the average required 44 per cent more nutrients per pound of milk when receiving the low-phosphorus ration. At this point attention may be called to the effect of low phosphorus upon reproduction, as pointed out in another section of this report. With an extremely deficient phosphorus ration many cows reproduce only once in two years in place of once each 12 months, as is generally considered desirable.

It is clear that the low phosphorus intake may bring about the high feed requirement noted by reducing the level of milk production. It is well understood that under practical conditions increasing the level of production results in a greater feed economy, chiefly because maintenance is essentially a fixed charge so that as the production level rises the proportion of nutrients used for this purpose increases. The possibility of a lower utilization of the feed nutrients in the body also exists but can not be judged from the figures given for these three cows.

Relation to Economy of Gain

Altho no definite experiments have been conducted to study the relation of phosphorus to feed requirements since one of our previous reports (4), considerable evidence relating to it has accumulated incidental to other phases of our experimental work. A summary of these new data, along with some of those included in a different form in our previous report, are presented in Table 22 and in more detail in Appendix tables 21 and 22.

The data included in Table 22 were obtained in connection with the study of the effect of magnesium sulfate on the phosphorus requirements of cattle. The results of this study are found in another section of this report. Three groups of Holstein heifers, ranging from 8 to 18 months of age at the beginning of the experiment, were used. All three groups were fed the same basal ration low in phosphorus. Group 1 received no supplements, Group 2 received a supplement of 150 grams daily of $MgSO_4 \cdot 7H_2O$, and Group 3 also received the magnesium sulfate supplement but in addition 65 grams of $NaH_2PO_4 \cdot H_2O$ daily. An attempt was made to maintain a uniform plane of nutrition for the animals in the three groups. However, there was a tendency, as is shown in Appendix table 21, for the animals on the low phosphorus rations (Groups 1 and 2) to consume less food toward the close of the trial, but all animals consumed considerably more nutrients than were required for maintenance. All animals received adequate amounts of protein. It is apparent that altho the experiment was not definitely designed for the purpose of determining the relation of the phosphorus intake to food utilization, the conditions of the experiment were such as to make such a use of the data possible.

Table 22

Nutrient and Mineral Intake in Relation to Gain in Live Weight of Growing Cattle

Group 1, phosphorus deficient ration; Group 2, phosphorus deficient ration plus $MgSO_4 \cdot 7H_2O$; Group 3, phosphorus deficient ration plus $MgSO_4 \cdot 7H_2O$; plus $NaH_2PO_4 \cdot H_2O$.

Group No.	Daily gain	Phosphorus intake daily	Total digestible nutrients	
			Per lb. of gain	Above maintenance per lb. of gain
	lb.	gm.	lb.	lb.
	Averages, first period, 174 days			
1	1.042	7.55	8.13	3.08
2	1.245	8.21	7.49	2.54
3	1.252	16.34	7.31	2.35
	Averages, second period, 240 days			
1	0.372	7.26	23.39	7.12
2	0.402	7.88	23.85	5.82
3	0.951	21.89	11.16	3.32

It will be observed that the experimental period of all the animals in Table 22 and Appendix table 21 is divided into two parts. This was considered necessary in order to determine the possible effect of a deficiency of phosphorus. These animals had previously received rations rich in phosphorus and for this reason probably had considerable amounts stored in their bodies. It is well known that the effects of a phosphorus deficiency in the ration do not become apparent until such time as this reserve becomes exhausted. The truth of this statement is indicated by the data. As will be observed, there is not a great difference between the three groups in the rate of gain and the amount of nutrients required per pound of gain during the first 174 days on experiment. Figure 4 shows clearly the greater effect of the phosphorus deficiency on the rate of gain near the close of the first period or after the phosphorus reserve of the body became largely depleted.

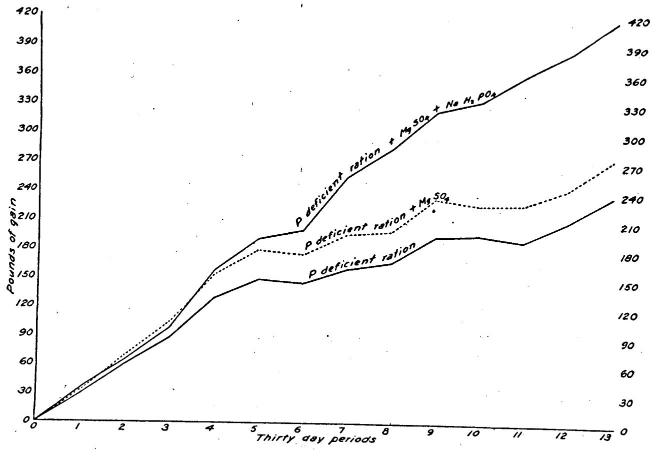


Fig. 4. Effects of Phosphorus Intake Upon the Rate of Growth of Heifers

The three groups received a basal ration deficient in phosphorus but supplying ample protein and digestible nutrients for normal growth. The group receiving a phosphorus supplement made a much more rapid gain in weight after the first four months.

The group receiving the phosphorus-deficient ration made an average gain of 0.6 pound; the group receiving the Epsom salt supplement 0.68 pound, and the one receiving the Epsom salt and sodium phosphate 1.06 pounds.

During the next 240 days the specific effect of the phosphorus deficiency in the ration became apparent, for not only was there a marked decrease in the rate of gain in weight in the two low-phosphorus groups, but these gains were made at considerably greater expense in nutrients than in the case of the animals provided with a more liberal phosphorus intake. It is apparent that the latter group also had a considerably better appetite, as indicated by the greater amount of nutrients eaten in excess of their requirements. This fact corroborates the statement made by many cattle owners in phosphorus-deficient regions, that their cattle have poor appetites. To the stockman this is a matter of considerable consequence inasmuch as the cost of the gains is determined in terms of the total amount of nutrients the animal receives instead of on the basis of the amount received over that required for maintenance. Considered in this way it will be observed in Table 22 that there is little difference between the three groups during the first period in the actual amount of nutrients required per pound of gain. However, during the second period Group 1, the check group on low phosphorus, used 23.39 pounds of digestible nutrients per pound of gain contrasted to 11.16 pounds by Group 3 with the phosphorus supplement. Expressed in percentages, Group 1 used 109 per cent more nutrients per pound of gain than Group 3.

That the difference is not to be explained entirely by the rate of gain is shown by the fact that after taking out estimated maintenance, 7.12 pounds of digestible nutrients were used by Group 1 to 3.32 pounds by Group 3 for each pound of gain in live weight. This shows that the nutrients consumed were used less efficiently by the body in the presence of a phosphorus shortage.

Figure 5 is a graphic representation of the data in Appendix table 22.

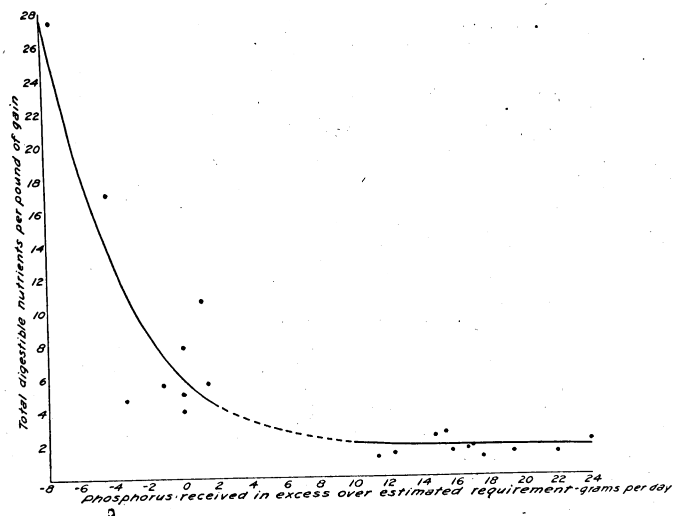


Fig. 5. Effect of Phosphorus Intake on Nutrients Required per Pound of Gain in Live Weight

These data are carefully selected from animals used in various other phosphorus deficiency studies carried on at this Station. In selecting the animals to be included care was taken to eliminate all those in which any factor other than the one considered might affect the results. All animals were mature or nearly so. Altho the plane of nutrition was not the same for all animals included, it was uniform for each animal during the period represented. In the case of lactating animals the first month or more following parturition was omitted to avoid including the extremely low weight that usually follows this phenomenon and also for the purpose of eliminating the individual variations in milk yield that frequently occur during the first few weeks following calving. Similarly, the two or three months immediately preceding parturition were omitted to avoid including the more rapid gain in weight that usually occurs during this period. Care was also taken not to include data from any animals in which the ration fed immediately preceding the experimental period might influence the reliability of the results. To place the milk yield of all lactating animals upon a comparative basis their daily production was converted to standard 4 per cent fat milk according to the formula of Gaines and Davidson:[6]

$$\text{Fat-corrected milk} = 0.4M + 15F$$

In which M stands for milk yield in pounds, and F for fat yield in pounds.

In order to have a basis for comparing the adequacy of the phosphorus provided in the rations, Armsby's estimate of the phosphorus required for maintenance (one gram of phosphorus per 100 pounds live weight) was used and, in addition, one gram for each pound of 4 per cent milk produced daily. Haecker's standard as given for milk of 4 per cent fat in Minnesota Bulletin 218[7] was used in determining the nutrient requirements for the milk produced.

The data in Appendix table 22 are arranged according to the amount of total digestible nutrients required per pound of gain, after deducting the amount estimated for maintenance and milk production. While it is probable that some of the variations exhibited are due to the individuality of the animal or result from unknown factors not eliminated in the selection of the animals, yet the fact that there is a very definite tendency toward a higher nutrient requirement per pound of gain in the animals having a low phosphorus intake seems quite significant, especially when considered in connection with the data in Table 22 above. It should be pointed out that the present data do not indicate that a supply of phosphorus in excess of a certain physiological optimum has any beneficial effect on the efficiency of utilization of nutrients. These figures merely bear out the results previously presented, that the animal uses food nutrients less efficiently when suffering from an insufficient supply of phosphorus than when an ample supply is available. A satisfactory explanation of this phenomenon is not yet known, altho we have been giving it special study. Apparently, the explanation does not lie in a lowered digestion coefficient.

RELATION OF PHOSPHORUS DEFICIENCY TO MILK PRODUCTION

As would be expected from the serious physical condition often observed, a phosphorus-deficient ration results in a low level of milk production. In Bulletin 229 and elsewhere (3), we have published data showing that the ash, calcium, and phosphorus concentration is within normal limits in milk produced on a low-phosphorus diet.

[6] Ill. Expt. Sta. Bull. 245.

[7] Eckles, C. H. Feeding the Dairy Herd. Minn. Agr. Expt. Sta. Bull. 218.

In regard to the quantity of milk produced, the reverse is found. Our first tests were to add a phosphate supplement to the basal ration (low phosphorus) after cows clearly suffering from phosphorus deficiency were partly through their lacation period. Decidedly favorable results were obtained (3). The curve of decline in milk production with the advance in lactation made a decided break at the point where a phosphate supplement was added to the ration. From this point on, the decline in milk production due to advance in lactation by the cows receiving the phosphorus supplement was below the normal for cows receiving ordinary rations.

It was recognized that data taken under these conditions have comparatively little value, and that there is a strong tendency for a lactating cow to follow the normal curve of decline in milk production during the lactation period. This means that her level of production during a lactation period bears a rather close relation to the starting level. If a cow, for example, having a capacity of 50 pounds of milk daily, as a result of poor physical condition or dietary deficiencies previous to parturition produces only 25 pounds of milk a day in the beginning of her milking period, by no later change in diet can her level of milk production be raised materially during the entire lactation period. It is understood by skilled managers of dairy cattle that when a cow has been improperly fed until her lactation period is partly past, a correction in the diet will not then restore her milk production to the level where it would have been with proper feed from the beginning. For these reasons it is clear that correcting the phosphorus deficiency in a ration after a cow has been in milk several weeks will not give entirely satisfactory evidence regarding the relation between an adequate phosphorus supply and milk production. In this case the experimental results would unquestionably be far below the real values.

As noted, experiments conducted by this general plan, that is, by adding phosphorus to a deficient ration after a cow was partly through her lactation, did show decidedly favorable results. It was recognized that the benefit found was probably minimized by the factors explained and that the only satisfactory plan would be to obtain results for complete lactation periods. With this in mind, cows were purchased in the low-phosphorus localities shortly before freshening. Those selected were from farms where troubles due to phosphorus deficiency were common. These cows were brought to University Farm and given good care and treatment. The ration was that typical for the farm from which the cows were purchased and which was known to be phosphorus deficient. The forage was low-phosphorus prairie hay in all cases. The cows selected were kept on this ration during a complete lactation period. Care was taken to supply sufficient protein and total digestible nutrients in accordance with Morrison's Feeding Standards. With certain animals a level was fixed somewhat above that of the feeding standard and the ration so apportioned as to approximate this level throughout the period of the experiment.

As soon as the cows reached the end of their lactation periods the feeding of sodium phosphate supplement was begun, the ration otherwise remaining the same. Two of the five cows received a $CaCO_3$ supplement during the lactation on the low phosphorus. The inorganic phosphorus in the blood plasma is also available for all lactation periods except the first for E 33. The data are given in Table 23. The term "level of nutrition" refers to the intake of nutrients in comparison with the requirement of the animal as estimated by Morrison's Feeding Standard. Altho considerable departure is shown by certain figures, it is clear in all cases that the total digestible nutrients were considerably in excess and it is inconceivable that the level fed was in any way responsible for the marked increase in milk production which accompanied the use of the phosphate supplement. Only one cow, E 33, was slightly below the standard in protein intake during the period of low phosphorus feeding. One other, E 94, was slightly below during the period of phosphorus supplement. It will be seen that the level of phosphorus intake was reflected clearly in the inorganic phosphorus in the blood plasma.

The ration used was limited to prairie hay and oats, the most common ration of the phosphorus-deficient area in Minnesota. However, with some of the higher producing cows it was found impossible to supply sufficient protein by the use of prairie hay and oats when milk production was at the highest level and for short periods small amounts of corn gluten meal were added to the ration. It was appreciated that the milk production might be limited by an inadequate amount of protein as well as by the low phosphorus. This possibility is further suggested by the results for the third lactation period for E 94. After obtaining the data for a lactation period from a low-phosphorus ration composed of prairie hay and oats, and a second from the same ration plus phosphorus supplement, she was continued for a third lactation using the hay of the same quality but using a mixed ration of concentrates in which cottonseed meal and wheat bran occupied an important part. The purpose was to supply a very liberal amount of phosphorus from the grain ration and determine if the blood phosphorus and milk yield could be maintained at a normal level by this means. The results were an increase in milk production, from 2,913 pounds on the low-phosphorus to 9,530 on the high-phosphorus ration. The level of protein feeding

Table 23

Relation of Phosphorus Intake to Milk Production

No. of animal	Character of ration	Production		Butterfat	Level of nutrition		Average mineral intake daily		Average inorganic phosphorus in 100 cc. blood plasma
		Milk	Butterfat		Digestible protein	Total digestible nutrients	Ca	P	
		lb.	lb.	per cent	per cent	per cent	gm.	gm.	mgm.
E 33	Low P	3,740	158.8	4.25	94.1	107.2	25.4	19.4	....
E 33	Low P plus $NaH_2PO_4 \cdot H_2O$....	8,658	401.2	4.63	100.5	111.5	27.9	48.2	5.73
	Percentage increase	131.5	152.6	....	....	....	....	148.5	....
E 75	Low P plus $CaCO_3$..........	3,276	140.2	4.28	103.3	120.1	58.6	15.0	1.83
E 75	Low P plus $NaH_2PO_4 \cdot H_2O$....	5,018	217.1	4.33	101.9	114.0	37.2	35.7	3.78
	Percentage increase	53.2	54.9	....	....	....	....	138.0	....
E 91	Low P	4,463	167.4	3.75	124.3	138.9	23.5	17.0	2.15
E 91	Low P plus $NaH_2PO_4 \cdot H_2O$....	6,714	256.9	3.83	102.5	110.1	25.0	43.5	6.20
	Percentage increase	50.4	53.5	....	....	....	....	155.9	....
E 93	Low P plus $CaCO_3$..........	3,543	134.9	3.81	99.6	123.9	64.7	14.2	1.42
E 93	Low P plus $Ca_3(PO_4)_2$.......	8,745	329.6	3.77	114.5	114.9	66.4	43.0	4.40
	Percentage increase	146.8	144.3	....	....	....	....	202.8	....
E 94	Low P	2,913	109.0	3.74	103.3	123.2	29.4	12.7	1.42
E 94	Low P plus $NaH_2PO_4 \cdot H_2O$....	5,015	170.8	3.41	97.9	120.4	23.3	39.2	5.55
	Percentage increase	72.2	56.7	....	....	....	....	208.7	....
E 94	High P, natural feeds.......	9,530	341.1	3.58	133.7	120.9	29.8	57.3	5.27

was increased by this ration to 133 per cent and the phosphorus intake to 57.3 grams per day compared to 12.7 grams on the low-phosphorus ration and 39.2 grams during the lactation period, when the phosphorus supplement was added. The total digestible nutrients in the ration were at approximately the same level in the three lactation periods. It is impossible to estimate how much of the greatly increased production during the third period should be attributed to the larger protein intake, the better quality of the proteins, or the greater phosphorus supply. The inorganic phosphorus in the blood plasma was normal throughout the lactation period. The increased milk production by all the cows during the periods of increased phosphorus intake is striking, the increases ranging from 50 to 146 per cent. In every case the animals also made a marked gain in weight and showed striking improvement in physical condition and vigor. One of the surprising results was the high level of milk production attained by E 33 and E 91, and to a less extent by all the others on a ration limited practically to prairie hay and oats. It can be accounted for only by the fact that sufficient nutrients were supplied and the phosphorus intake was high enough to meet the demands of the productive capacity of the animal.

The calcium intake was relatively low except when $CaCO_3$ was supplied as a supplement. Mineral balance trials were not made of the animals during the periods covered by these data, hence it is impossible to judge as to the possibility of loss of calcium during the periods represented. Judging from the results of balance trials conducted by others, it is not unlikely that calcium losses occurred, at least during the early part of some lactation periods. However, for ordinary production, the calcium supply appears to be sufficient so far as can be judged by long-time observations of these and other animals receiving similar rations. Data from balance trials are available for other cows under similar conditions. (See Appendix tables 7, 8, 9, and 5.) E 92, with a daily milk production of 26.3 pounds and a calcium intake of 26.56 grams, had a daily negative balance of 3.97 grams. E 74, producing 19.6 pounds of milk daily, had a daily negative balance of 7.09 grams of calcium with a daily intake of 24.6 grams. E 94, dry, with a daily intake of 25.5 grams, stored 3.11 grams per day. The probabilities are that the cows supplying the records in Table 23 were in a condition of negative calcium balance in the first part of their lactation period and were able to gain this back when a low level of production was reached.

The most significant evidence that the phosphorus intake was too low during the lactation periods without any phosphorus supplement is the low inorganic phosphorus in the blood plasma. This condition was in evidence with all these animals when lactation began, and continued at about the same level or with some decline as lactation progressed. There can be no question that the increase in milk production during the lactation periods when phosphorus supplements were used was the result of the increased phosphorus supply. It is true that the cows received a more liberal ration during the lactation periods having the higher phosphorus intake. However, the ration was adjusted to the level of milk production in both periods and care was taken to have the feed intake at such a level that the supply of protein and total digestible nutrients would at no time be factors in limiting the amount of milk produced.

Further indication that the phosphorus intake was the limiting factor during the lactation periods with the low phosphorus intake is shown by comparing the phosphorus received with the estimated requirements. Armsby's (40) figures for maintenance and milk production may be used as a basis for such a comparison. The figures which follow are prepared in this manner, using the data for the 30-day period during which milk production was at the highest point.

	Milk	Estimated phosphorus requirement	Phosphorus intake
	lb.	gm.	gm.
E 75			
Low phosphorus	3,276	18.6	16.9
Phosphorus supplement	5,018	21.8	31.4
E 91			
Low phosphorus	4,463	19.5	17.8
Phosphorus supplement	6,714	27.7	46.3
E 94			
Low phosphorus	2,913	16.4	14.1
Phosphorus supplement	5,015	28.7	39.3
E 93			
Low phosphorus	3,543	16.8	13.9
Phosphorus supplement	8,743	30.7	47.8

It is recognized that the figures are subject to large probable errors but they indicate that the phosphorus intake was a limiting factor in milk production during the lactation period in which the low milk production was obtained.

The economic importance of supplying the needed phosphorus in the rations of dairy herds maintained on farms located on phosphorus-deficient soil is evident. In Minnesota alone, phosphorus-deficiency symptoms have been observed among dairy cattle in thirty counties. In badly affected districts trouble occurs at times on nearly all farms; in other districts on only a few, owing to either a lower soil phosphorus or poorer feeding methods on these particular farms. Our results as set forth in Table 23 suggest that on farms where serious phosphorus deficiency exists, even when sufficient home-grown feeds are supplied to furnish the digestible nutrients needed, the milk production may be limited to a low level. The five cows used in the experiments reported averaged 3,587 pounds of milk in a lactation period. During the next period, receiving the same feeds, fed as before in proportion to milk production but with an adequate phosphorus intake, the same cows averaged 6,830 pounds of milk, an increase of 80 per cent.

These statements should not be interpreted to mean that increased milk production will necessarily follow the use of supplements carrying phosphorus and that such supplements are recommended for general use. There is no evidence now available that the addition of phosphorus-bearing mineral mixtures will have any effect whatever on the level of milk production on farms where the soil is well supplied with phosphorus and the forage is consequently not abnormally low in this element.

RELATION OF MAGNESIUM SULFATE INGESTION TO PHOSPHORUS DEFICIENCY

In our first report regarding phosphorus deficiency, mention was made of the high sulfate content of the water in the section of the state where the trouble occurs. Samples of water taken on 26 farms reporting phosphorus deficiency were found to contain an average of 220 parts of SO_4 per million (1). Water from many wells in this area contains so much magnesium sulfate that it has a decidedly bitter taste. Having in mind the so-called "calcium magnesium antagonism" we were inclined to consider the intake of such large quantities of magnesium sulfate a factor that might have a decided significance in the deficiency problem. In our first experiment, which was an attempt to produce the symptoms in experimental animals, we added Epsom salt to the drinking water received by the subjects in amounts to equal the magnesium sulfate content of the water on one of the farms where for years trouble had been common. The ration for all animals was limited to feeds grown on a farm where trouble regularly occurred. Part of the group received a supplement of sodium phosphate, and others of calcium carbonate. The results were that the animals receiving the phosphorus supplement were the only ones that did not develop symptoms typical of those which appeared in the herds experiencing the trouble, which first lead to the study. This experiment did not clear up the question as to the possible importance of the sulfate.

Later, a second group of cows showing marked symptoms of mineral deficiency were brought from farms in the affected area, together with the feed they had been consuming. One pair received sodium phosphate as a supplement, a second pair tricalcium phosphate, a third pair calcium carbonate, one animal sodium phosphate plus magnesium sulfate, another tricalcium phosphate plus magnesium sulfate. All those receiving the phosphate supplements rapidly lost all symptoms of deficiency regardless of the intake of magnesium sulfate. The results to this point did not indicate the magnesium sulfate to be a factor in causing the deficiency symptoms. However, accumulation of additional data showing a high magnesium sulfate content of the water supply is general in the region affected by phosphorus deficiency served to maintain interest in this possible factor. To obtain further evidence, mineral balance trials were conducted in which certain animals received magnesium sulfate supplements. The results indicated some effects in the way of negative balances of calcium and phosphorus when an excessive amount of the sulfate was fed. However, the results were inconsistent and not conclusive and a series of more comprehensive experiments was planned. It was decided to conduct a long-time feeding experiment combined with mineral balances made after the animals had been subject to the particular ration for some months.

Nine healthy, well bred Holstein heifers were chosen, ranging in age from 8 to 15 months. They were divided into three groups to be fed as follows:

Group 1 (E 123, E 120, E 125). A check group to receive a ration (later referred to as the basal ration) low in phosphorus with no mineral supplements.

Group 2 (E 118, E 121, E 122) to receive the same low phosphorus ration as Group 1 with the addition of 150 grams daily of Epsom salt.[8]

Group 3 (E 124, E 126, E 127) to receive the same basal ration and 150 grams of Epsom salt and 65 grams of $NaH_2PO_4 \cdot H_2O$ daily.

The basal ration consisted of prairie hay grown on low-phosphorus soil, a grain mixture selected to provide a relatively low phosphorus intake, and black strap molasses (from cane sugar manufacture). Common salt was provided *ad libitum*. The Morrison standard for nutrient requirements of growing animals served as a guide in calculating the amount each animal should receive. The animals were housed in the dairy barn, where they were tied in ordinary stalls, and turned outside for exercise in a dry lot. Water was supplied in drinking cups. Blood samples from each animal were drawn three days in succession each month. From the three daily samples a composite sample was prepared

[8] The Epsom salt used throughout this experiment contained approximately 10.7 per cent magnesium instead of the theoretical 9.866 per cent in $MgSO_4 \cdot 7H_2O$.

and the calcium and inorganic phosphorus determined in the blood plasma. The details of nutrient and mineral feed intake and blood analyses are found in Appendix tables 23 to 31 and the composition of the feeds used in Appendix table 43.

Table 24 shows a summary of the daily average calcium and phosphorus intake and the calcium and inorganic phosphorus in the blood plasma by groups for the 30-day periods, numbers 1 to 6, 7 to 10, and 11 to 14.

Table 24
Mineral Intake and Blood Analyses—Epsom Salt Experiment

	Daily Ca intake	Daily P intake	Daily Mg intake	In 100 cc. blood plasma	
				Ca	Inorganic P
	gm.	gm.	gm.	mgm.	mgm.
Group 1, check					
Periods 1– 6	23.91	7.55	14.19	10.93	5.30
Periods 7–10	23.27	7.27	10.77	11.60	3.10
Periods 11–14	36.34	7.24	15.79	10.94	3.11
Group 2, check ration plus Epsom salt					
Periods 1– 6	25.84	8.22	24.12	10.60	5.52
Periods 7–10	26.22	7.99	26.62	10.89	3.67
Periods 11–14	40.93	7.78	32.41	10.97	3.15
Group 2, check ration plus Epsom salt and $NaH_2PO_4 \cdot H_2O$					
Periods 1– 6	25.79	16.08	23.90	10.61	6.44
Periods 7–10	28.56	22.80	27.72	10.50	5.14
Periods 11–14	47.46	20.97	35.08	9.89	5.84

Table 24 indicates that the calcium intake was fairly uniform for the three groups. The phosphorus intake was practically the same for Groups 1 and 2 and more than double for Group 3. The magnesium intake, owing to the Epsom salt supplement, was high for Groups 2 and 3.

The calcium content of the blood plasma was essentially the same for the three groups. The inorganic phosphorus content of the blood plasma was normal for all groups during the first five periods. Certain individuals in Groups 1 and 2 showed a slight drop in blood plasma in Period 6 but too small to be conspicuous in the average for the first six periods.

All animals in Groups 1 and 2, receiving no phosphorus supplement, showed a marked drop in blood phosphorus during Period 7 and remained at essentially the same level during the seven periods which followed. This fact may be observed in Table 24 and in the detailed data found in Appendix table 32. Group 3, receiving the phosphorus supplement in addition to the Epsom salt, remained normal in blood phosphorus throughout the 420 days of the experiment. The chemical composition of the humerus from one animal of each group is given in Table 25 and the appearance of these bones is shown in Figure 6.

Table 25

Composition of Humerus of E 123, E 122, and E 126 in Magnesium Sulfate Experiment

Cow No.	Age	Weight of green bone	Ash in bone			$\frac{Ca_3(PO_4)_2}{CaCO_3}$ ratio of minerals
			Green	Dry	Dry extracted	
	months	gm.	per cent	per cent	per cent	
E 123............	24	1,119	25.6	33.4	61.6	5.38
E 122............	29	1,117	30.2	37.4	62.3	5.94
E 126............	29	1,250	37.5	43.9	66.3	7.25

The first indications of pica, a desire to chew bones, were noticed for animals in Group 1 near the end of the sixth period, at the time the blood phosphorus began to decline. Those in Group 2 exhibited the same symptoms a little later. Other characteristic symptoms of phosphorus deficiency were observed later with both Groups 1 and 2, especially some lack of appetite for hay and a generally poor physical condition. They walked with a shuffling gait and when standing drew their feet rather far under them, both in the rear and in front. There was some indication of the head appearing too large for the body, as seen among animals in the phosphorus-deficient regions. This was especially noticeable with E 123. Group 3 was in excellent condition throughout the entire experimental period of 420 days. Differences between the groups in physical condition and well being were reflected in the rate of gain. From October 13, 1928, to November 7, 1929, the average gains by groups were as follows:

	Character of ration	Total gain	Gain per day
		lb.	lb.
Group 1	P deficient	236	0.60
Group 2	P deficient plus Epsom salt......................	276	0.68
Group 3	P deficient plus Epsom salt and sodium phosphate..	414	1.06

These data are shown graphically in Figure 4, and the condition of the animals after 11 months on experiment in Figure 7.

The striking difference in the gains by Group 3 and Groups 1 and 2 is in line with our previous results and with observations made during the survey of the low-phosphorus soil area. It also agrees with the statement so often made by farmers in the low-phosphorus area that their cattle were always in poor flesh and low physical condition regardless of how they are fed. The same results have been observed by Theiler and associates (13, 41). The wide difference in the rate of gain apparently is due chiefly to the better consumption of feed, especially roughage by Group 3, receiving the phosphorus supplement, as well as to its better utilization.

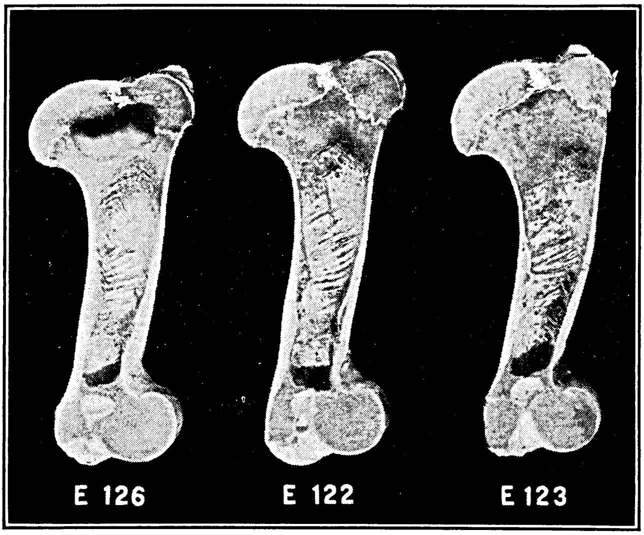


Fig. 6. Longitudinal Half Sections of Right Humerus of E 122 After Receiving a Low-Phosphorus Ration for 12 Months; of E 123 After the Same Period on a Similar Ration Plus 150 Grams Daily of Epsom Salt; and of E 126 After Receiving a Similar Ration as E 123 Plus 65 Grams Daily of $NaH_2PO_4 \cdot H_2O$ for the Same Period

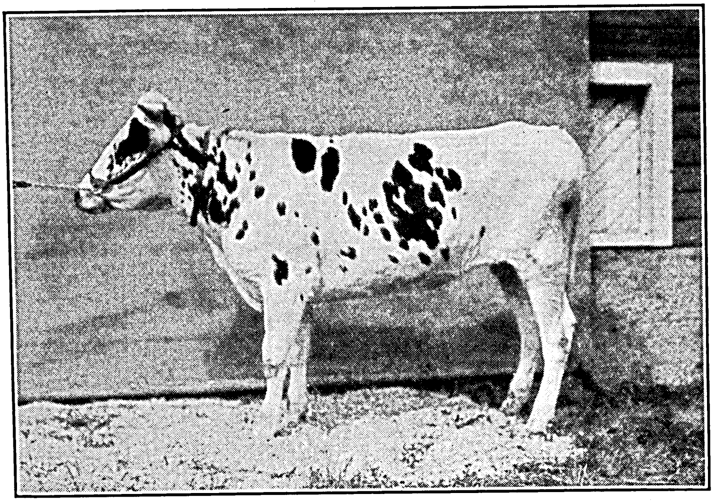
It is to be noted that the Epsom salt feeding did not produce a more severe condition of the bones on the low-phosphorus ration in the case of E 123 and did not prevent the formation of a dense, heavily mineralized bone in the case of E 126, which received the phosphate supplement.

Balance Trials

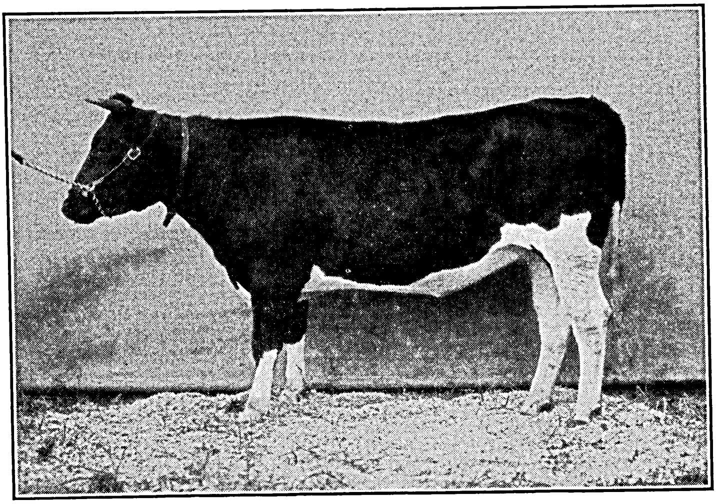
The purpose of the experiments here reported was to determine if the relatively large intake of magnesium sulfate in the drinking water is a factor in causing the mineral deficiency troubles as experienced in western Minnesota. Certain balance trials (5) of a preliminary nature gave some support to the theory of "calcium magnesium antagonism." In the experiments as now reported, the problem was approached from two angles, long-time feeding experiments and balance trials.

After the three groups had been on the experimental rations for about eight months, mineral balance trials were conducted for E 120 in the check group, E 121 and E 122 in the group receiving Epsom salt, and for E 124 in the group receiving both Epsom salt and sodium phosphate. Again, after about a year from the beginning of the experiment, a second mineral balance was conducted for each of these animals.

Irregularities in the amount of common salt consumed had attracted our attention and, in order to determine if it was a factor, a third mineral balance was conducted for E 124 and E 121 during which the intake of salt was limited to one ounce per day.



E 123



E 127

Fig. 7. Condition of Representative Animals After Eleven Months on Epsom Salt Experiment E 123, on phosphorus-deficient ration, average daily gain 0.67 pound. E 127, on phosphorus-deficient ration plus Epsom salt plus sodium phosphate, average daily gain 1.13 pounds. Both animals received the same percentage of digestible nutrients above estimated maintenance requirements.

Detailed results of the ten balance trials are given in Appendix tables and are summarized as follows:

Summary of Balance Trials

E 120 Check group. Low P ration for 236 days; blood plasma, Ca 11.80 mgm., inorganic P 3.61 mgm. per 100 cc. (Appendix table 33).

	Ca gm.	P gm.	Mg gm.
Intake per day	33.06	8.18	14.56
Balance per day	+2.56	+1.53	−1.20

E 120. Low P ration for 360 days; blood plasma, Ca 10.50 mgm., inorganic P 4.29 mgm. per 100 cc. (Appendix table 34).

	Ca gm.	P gm.	Mg gm.
Intake per day	36.58	8.08	14.06
Balance per day	+0.77	+0.23	−5.43

E 121. Low P ration, plus Epsom salt for 251 days; blood plasma, Ca 10.0 mgm., inorganic P 3.75 mgm. per 100 cc. (Appendix table 35).

	Ca gm.	P gm.	Mg gm.
Intake per day	34.64	9.08	35.76
Balance per day	−1.03	+1.07	+1.70

E 121. Low P ration, plus Epsom salt for 379 days; blood plasma, Ca 10.50 mgm., inorganic P 4.29 mgm. per 100 cc. (Appendix table 36).

	Ca gm.	P gm.	Mg gm.
Intake per day	38.54	8.55	31.07
Balance per day	−2.51	−0.47	−1.41

E 121. Same ration as in previous with salt (NaCl) limited to one ounce daily (Appendix table 37).

	Ca gm.	P gm.	Mg gm.
Intake per day	36.99	8.01	31.27
Balance per day	−1.60	−0.27	−0.61

E 122. Low P ration, plus Epsom salt for 236 days; blood plasma, Ca 12.0 mgm., inorganic P 4.08 mgm. per 100 cc. (Appendix table 38).

	Ca gm.	P gm.	Mg gm.
Intake per day	33.21	8.09	30.08
Balance per day	−0.76	+0.32	−1.68

E 122. Same ration as before for 360 days; blood plasma, Ca 10.50 mgm., inorganic P 3.85 mgm. per 100 cc. (Appendix table 39).

	Ca gm.	P gm.	Mg gm.
Intake per day	37.03	8.16	30.28
Balance per day	+0.36	+0.87	−2.76

E 124. Low P ration, plus Epsom salt and sodium phosphate for 251 days; blood plasma, Ca 11.25 mgm., inorganic P 5.18 mgm. per 100 cc. (Appendix table 40).

	Ca gm.	P gm.	Mg gm.
Intake per day	31.24	23.71	35.76
Balance per day	+2.34	+3.67	+2.85

E 124. Low P ration, plus Epsom salt and sodium phosphate for 379 days; blood plasma, Ca 9.85 mgm., inorganic P 5.05 mgm. per 100 cc. (Appendix table 41).

	Ca gm.	P gm.	Mg gm.
Intake per day	44.75	24.29	33.75
Balance per day	+3.01	+3.69	—5.90

E 124. Ration as in previous trial with salt (NaCl) limited to one ounce daily (Appendix table 42).

	Ca gm.	P gm.	Mg gm.
Intake per day	46.18	23.85	34.93
Balance per day	+1.11	+2.74	—3.78

We are unable to conclude from the data given that a high intake of magnesium sulfate is a factor in producing phosphorus deficiency in cattle. Group 2, with a magnesium intake nearly double that of Group 1, did not develop symptoms of phosphorus deficiency any sooner, in fact, not quite so quickly as did Group 1. Both groups showed the same symptoms when they did develop and the blood phosphorus was not noticeably different.

The balance trials also failed to develop any significant differences between the groups. It is true that with six mineral balances in which the ration included a supplement of Epsom salt, the calcium was negative in four and positive in two. However, the largest negative balance, 2.51 grams of Ca, is only 6.2 per cent of the intake. As is well known, the experimental error in conducting balance trials is considerable. It is probable that the variation between the individual results for E 120 and E 121 and E 122 are no greater than had the three trials been made in succession with the same animal. The phosphorus balance was positive in five out of seven trials on the low P ration and no evidence appears that the larger magnesium intake for Groups 2 and 3 was a factor. Cow E 124, receiving both Epsom salt and sodium phosphate, gave positive balances in both calcium and phosphorus in all three trials. It is difficult to interpret the negative magnesium balances that were in evidence in 8 out of the 10 trials. Limiting the intake of sodium chloride had no significant effect upon the retention of the three minerals.

The question may be raised concerning the development of typical phosphorus deficiency as shown by physical symptoms and blood analyses when balance trials show either a small positive balance or a negative one too small to be significant. The explanation apparently is that the skeletons of these animals were in a period of rather rapid growth and the demand for calcium and phosphorus for this reason was relatively large. It is evident that deficiency symptoms may develop with a growing animal regularly showing a small positive balance. The retention of from 2.74 to 3.69 grams of phosphorus daily by E 124 may be taken to indicate, in general, the amount that was used in the skeleton of this growing animal.

RELATION OF PHOSPHORUS DEFICIENCY TO REPRODUCTION

In our earlier publication observations were reported regarding the effect of phosphorus deficient rations upon reproduction in cows. It was found in the survey of affected areas that reproductive troubles were common, the most frequent being irregularities in the oestrum periods. In some herds the calf crop was not more than 50 per cent, following dry seasons. Many farmers testified that it was a common experience for cows for dairy use to reproduce only once in two years, and that this experience was most common with the heaviest producing cows. Considerable material has been gathered on this subject during our experimental work, altho what has been obtained is a by-product from experiments set up with other objectives. We have found that a dairy cow which has for a considerable period received a ration short in phosphorus will usually show oestrum once or twice at about the normal intervals following parturition. If served, she will conceive in approximately the normal percentage of cases. If she does not become pregnant at this time, oestrum may not again appear during the entire milking period, if the low-phosphorus ration is continued. After she has been dry for a few weeks oestrum again appears and about the usual proportion of services results in conception. As a result of this condition there is a distinct tendency for cows on a phosphorus-deficient ration to reproduce once each two years. No tendency has been observed for a low-phosphorus intake to result in abortion. Only one cow aborted among a considerable number so fed as to suffer from phosphorus deficiency and she was positive to the blood test for Bang's disease.

Information received by correspondence from India indicates that reproduction once in two years is common among cattle in certain sections of that country and other evidence available suggests strongly that the cattle are subject to a serious phosphorus deficiency. DuToit and Bisschop (41) are responsible for the statement that the native breed of cattle in South Africa, the Africander, on the open range commonly reproduce once in two years. These investigators interpret this unusual condition to be the result of a mineral deficiency in the pastures. Tuff (39) states that in certain sections of Norway where mineral deficiency is especially severe, during years when this trouble is particularly prevalent, for example, following a dry season, a large number of animals, especially heifers, show no signs of oestrum during late winter and spring. Theiler and associates (42), after studying the value of feeding bonemeal to cows in the section of South Africa where phosphorus deficiency is very serious, report "Of the cows receiving the bonemeal, 80 per cent calved normally, . . . of the control cows, only 51 per cent (36 out of 70) calved." In another experiment in the same low-phosphorus region DuToit and Bisschop (41) report that of 109 cows receiving bonemeal in a three-year experiment, 66 per cent had three calves, while of 20 control cows surviving without phosphorus not one had three calves. The cows fed bonemeal produced 87 per cent of the possible number of calves, while the control group produced only 56 per cent. The authors concluded that the phosphorus reserves become depleted by gestation and lactation and that one or more barren years are necessary to build up the reserves to a point where pregnancy will take place. Hart and Guilbert (14), studying the calf crop in range herds in California, came to the conclusion that low mineral intake is one of the important factors causing the very low percentage calf crop experienced at times on certain ranches. They also give some significant results from the feeding of bonemeal to range herds. Guilbert and Hart (43) tested the effects of different calcium and phosphorus intakes on the oestrus cycle and reproduction of rats. They conclude "Diets containing 0.18 to 0.22 per cent phosphorus with a calcium to phosphorus ratio of approximately 4:1 fed to rats from sexual maturity to about 100 days of age caused ovulation to be irregular or to cease entirely. This level of phosphorus, however, appears to be adequate to maintain normal oestrus cycles (when begun) after about 110 days of age." Further, "There appears to be a relation between low phosphorus content of the blood and irregular ovulation history, but more data will have to be secured before this can be definitely concluded."

These observations and conclusions from experimental work confirm our observation and experience as given. However, we consider it desirable to obtain under carefully controlled conditions further evidence before arriving at a conclusion as to the importance of low phosphorus as a factor in reproduction troubles of cattle. We now have under way an experiment with a group of dairy cows with the special objective of obtaining data on this subject under properly controlled conditions.

PRACTICAL CONSIDERATIONS IN THE PREVENTION AND TREATMENT OF PHOSPHORUS DEFICIENCY

For livestock owners confronted with phosphorus deficiency among their animals three courses are open: (1) feed a ration that will supply the needed phosphorus in the concentrates used; (2) add a phosphorus-bearing supplement to the regular ration; (3) fertilize the soil with phosphate to such an extent that the forage grown will supply the needed amount of this mineral.

The livestock owner confronted with evidences of phosphorus deficiency among his animals will necessarily direct his attention to the first or second possibility and at the same time he may well consider the advisability of using phosphorus-carrying fertilizers in order to prevent the recurrence of the trouble in following years. In general, forages are low in phosphorus and relatively high in calcium, while for seeds including cereals and corn, the reverse is true. Furthermore, while the mineral content of forages varies widely with the available mineral content of the soil the mineral content of seeds and grains such as the cereals and corn appears to be more uniform regardless of the soil on which they were grown. With grains the result of a low level of available minerals, phosphorus for example, is reflected primarily in the yield rather than in the composition. An apparent exception to this is when such crops are grown on fertilized peat soils.

The following figures taken for the forages from Appendix tables 15 to 20 and for the concentrates from average analyses show the relative content in grams for 100 pounds:

	In 100 lb. calcium, gm.	In 100 lb. phosphorus, gm.
Prairie hay, P deficient soil	199	48
Alfalfa hay, market supply	630	95
Timothy hay, market supply	145	65
Corn	6	136
Oats	45	160
Wheat bran	29	584
Cottonseed meal	118	528
Linseed meal	165	336

These figures show the relatively large amount of phosphorus supplied by wheat bran, cottonseed meal, and linseed meal as compared to roughages or even by corn and oats. It is clear that the use of one of these high-phosphorus concentrates as a portion of the ration will go far in making up the deficiencies of low-phosphorus roughage. Alfalfa or other legume hay carries a much more liberal supply of phosphorus than either timothy or prairie hay. The yields of forage crops of this class are quite susceptible to the effects of a low level of available phosphorus in the soil and respond in a marked degree to the use of phosphate fertilizers on such soils. We have recommended the feeding of bonemeal as a safe and efficient means of relieving phosphorus deficiency and the practice has been adopted by large numbers of livestock owners. The product to be used is that prepared especially for livestock feeding. Its freedom from disease germs is insured by the high temperatures employed in its preparation. The animals should be allowed to consume as much of this product as their appetites demand. This is best done by supplying it in a box to which the animals have access. Cattle in need of phosphorus will usually eat it readily if it is of the proper grade, which is almost entirely free from odor. The mixing of bonemeal with salt is a common practice but the advantages seem to be in favor of feeding it separately. When mixed with salt the cattle may sometimes consume the mixture when phosphorus is not needed in order to get the salt, and again they may consume more salt than is desirable in order to get the amount of bonemeal called for by their appetites. The amount of bonemeal needed for an animal during the year will vary with the size, age, and milk production of the individual, the reserve supply in the body, and the intake provided by the ration used. Our experiments in supplying bonemeal to five herds in the low-phosphorus area showed 40 to 70 pounds per head was consumed during the year.

Largely as a result of our experiments, prepared mineral feeds are sold extensively in phosphorus-deficient regions in Minnesota. Most of these, if fed in large enough quantities, relieve the abnormal conditions resulting from a shortage of phosphorus. The results are due to that portion of the mixture, chiefly bonemeal, sometimes boneblack, which contains phosphorus. The questions that arise regarding these mixtures concern the nature of the ingredients and the price. The fairest comparison in price is with bonemeal on the basis of the phosphorus content.

When cattle are suffering from a lack of phosphorus there is no object in feeding a mineral mixture containing large quantities of lime and common salt. In fact, results from experimental work indicate that including lime in the form of limestone or marl under these conditions actually makes the trouble worse. Some mineral mixtures also contain rock phosphate, which is dangerous if present in any considerable quantities on account of its fluorine content. Neither are mixtures containing a great variety of ingredients desirable, for most of the constituents are usually valueless to an animal because no ordinary ration is likely to be lacking in them.

In looking for a source of phosphorus less expensive than bonemeal, rock phosphate at once comes into consideration. Our investigations have not included a study of the use of this product. It is clear from the work of Reed and Huffman (44, 37) that rock phosphate is a questionable and usually a dangerous product to use on account of the serious effects resulting from the presence of fluorine. So far data are not available upon which to base judgment as to the amount of fluorine an animal can tolerate. Presumably at least a small proportion of the phosphorus could be supplied in the form of rock phosphate without danger, but until the necessary information is at hand and a supply of rock phosphate available with a certain, as yet undetermined, minimum fluorine content, it appears unwise to use this product.

The third possibility suggested is the use of phosphorus-bearing fertilizers. It is well established that the phosphorus content of forages bears a close relation to the available phosphorus in the soil. As phosphorus deficiency with livestock appears only when receiving forage grown on a low-phosphorus soil, it is evident that one means of preventing the appearance of this trouble is the use of suitable phosphate fertilizers. On the experimental plots at Golden Valley, Minnesota (34), the phosphorus content of the dry matter in timothy was increased from 0.10 per cent on the unfertilized plots to 0.14 on plots receiving phosphate fertilizer. With brome grass and western rye grass the increase was even more marked.

Similar data are reported by Nygard, of the Montana Experiment Station (35). The first cutting of alfalfa on three fields averaged 0.14 per cent of phosphorus without fertilization compared to an average of 0.203 per cent on plots receiving phosphate fertilizer. In another county no significant increase in phosphorus content followed the application of phosphate fertilizers.

Many similar results are found in the literature. Mather (45), obtaining his material in Minnesota and Manitoba, reports an extended study of the effect of fertilizers upon the forms of phosphorus and the amount of phosphorus in hays. He found the amount of fertilizer needed to produce the maximum yield of hay per acre was far below that needed to produce a relatively high phosphorus content in the hay. He concluded: "It would seem, therefore, that phosphate fertilizer could be profitably applied only in so far as it increased the pounds of hay per acre, and that phosphate required in a ration in excess of the quantity supplied in such a hay could be added directly to the feed."

DuToit and Green (46), in South Africa, compared dicalcium phosphate with bonemeal by feeding the two in different quantities over a period of two years to groups of eight animals each. The animals were grazed according to practices employed in their locality on low-phosphate soil typical of that region. The animals receiving three ounces (containing 8.2 grams phosphorus) of bonemeal daily gained 134 per cent in weight compared to a gain of 127 per cent by the group fed two ounces daily of the dicalcium phosphate (containing 10.6 grams phosphorus). A group fed one ounce of bonemeal (12.7 grams phosphorus) gained 110 per cent in weight, and a group fed two-thirds ounce of dicalcium phosphate (3.5 grams phosphorus) gained 124 per cent in weight. Check animals receiving no supplement gained 42 per cent. It is apparent from these results that no marked distinction can be made between bonemeal and dicalcium phosphate on the basis of the effectiveness of equal quantities of phosphorus ingested to stimulate growth of young cattle.

DuToit and Green also attempted to determine the relative value of the two products to prevent osteophagia (bone chewing). While they conclude that their estimations "seem to show that weight for weight, calcium phosphate is three times as effective as bonemeal," the inaccuracy of the method employed is admitted, making these results unreliable. The probabilities are that the phosphorus in the two forms are equally effective, weight for weight, in preventing pica. The problem of the relative value of the two products, therefore, becomes solely one of the most economical sources of phosphorus. Since ordinary bonemeal contains 7.5 to 9.0 per cent phosphorus and commercial dicalcium phosphate 15 to 18 per cent phosphorus, the latter product could sell for twice that of the former and still be as economical a source of the necessary element. It should be pointed out, however, that dicalcium phosphate can not be self-fed satisfactorily because cattle will not eat it of their own accord. It must be fed in doses or mixed with the feed.

The economical as well as the practical aspects of feeding other forms of commercial phosphates are essentially the same as those applying to the comparison between bonemeal and commercial dicalcium phosphates. The cost should be on the basis of the amount of phosphorus purchased per pound of substance. The problem of palatability and ease of administration must also be taken into account. Finally, the product must not contain any foreign ingredients that will be toxic to the animals.

SUMMARY AND CONCLUSIONS

1. Six years of investigation of the phosphorus deficiency occurring among cattle in Minnesota since our first report in Minnesota Station Bulletin 229, in 1926, have confirmed all the major conclusions found in the former report and extended our knowledge in a number of important particulars.

2. As the result of these investigations, it is now known that phosphorus deficiency is more or less prevalent in 30 counties of Minnesota.

3. The occurrence of phosphorus deficiency is more widespread throughout North America than previous knowledge indicated. In addition to the localities mentioned in previous reports, it is now evident that the trouble may occur in Wisconsin, North Dakota, South Dakota, Colorado, Utah, Nevada, New Mexico, California, and Florida in the United States; and also in Manitoba, Canada.

4. Bone chewing is a specific symptom of phosphorus deficiency in its early and major stages, but disappears, together with pica in general, in advanced cases of long standing. The animal then becomes inert.

5. Mineral balances conducted with dry and lactating cows on low-phosphorus rations show that phosphorus deficiency is not necessarily accompanied by marked negative phosphorus balance unless the cow's body is being drained of phosphorus by copious milk flow. On the other hand, dry and lactating cows will show marked phosphorus storage in balance experiments when phosphate supplements are added to phosphorus-deficient rations.

6. Phosphorus deficiency of any desired stage of severity may be produced experimentally in young or mature or milking cows by limiting their phosphorus intake by means of suitable rations low in phosphorus. A detailed description is given in this bulletin of typical experimental aphosphorosis in cattle, showing how rapidly the symptoms develop under various experimental conditions, and pointing out the identity of the disease with that occurring under natural conditions in Minnesota.

7. The concentration of inorganic phosphate in the blood plasma in cattle aphosphorosis is an important index of the severity of the disease, as first pointed out by Palmer and Eckles (2) and later verified by the South African workers (18, 19). These data are presented in this bulletin, together with additional data showing (a) the great rapidity with which phosphate feeding causes a recovery from depleted blood phosphate, (b) the rapidity with which the concentration falls in aphosphorosis at different levels of phosphorus deficiency, when other influencing factors are equal, (c) the extent to which milk production decreases blood phosphate concentration in aphosphorosis, and (d) the

detrimental influence of a high calcium level in the ration on the rate of fall of blood phosphate during phosphorus deficiency.

8. Blood composition may be employed for the diagnosis of aphosphorosis in the field. Examples of this are given.

9. Large fluctuations in the concentration of inorganic phosphate in the blood plasma of cattle may occur from day to day. A study of some of the physiological factors which have been suspected of causing these variations is summarized.

10. Aphosphorosis in cattle produces marked effects on the mineral composition of the bones. In the fresh bone, the primary effects are the marked reduction in ash and its replacement by lipid material. These effects are magnified when the composition of the moisture-free bone is examined. When the moisture is driven off and the lipids are extracted, the aphosphorotic bones, especially the long bones (femur, humerus, ribs) are characterized by a low total ash and a lower $Ca_3(PO_4)_2$ and a higher $CaCO_3$ content than normal bones. The result is an abnormally low $Ca_3(PO_4)_2:CaCO_3$ ratio, such as is obtained in rickets in laboratory animals. This seems to be a specific effect of the phosphorus deficiency.

11. Two experiments comparing sodium acid phosphate and the phosphate forms in natural foods in relieving phosphorus deficiency and bringing about recovery, in general indicate no striking difference between these methods of administering phosphorus. Some differences in favor of the inorganic phosphate used were evident but the experiments were not intended as a demonstration of the practical value of the particular form of mineral phosphate employed. Furthermore, they can not be interpreted as having demonstrated the superiority of all forms of mineral phosphate over natural food sources of phosphorus.

12. The primary cause of phosphorus deficiency among cattle in Minnesota is revealed in the analysis of hay from grasses (prairie and timothy) grown in the areas where the trouble occurs. When cattle are obliged to subsist chiefly on hays whose phosphorus content is consistently as low as that shown in the data presented in this bulletin, they can not ingest enough phosphorus to meet their requirements. While data presented show that a higher annual rainfall increases the phosphorus of the grass hays in these regions, and thus causes a decrease in the severity of the outbreaks of phosphorus deficiency, the phosphorus content is still too low to insure optimum nutritive conditions.

13. When a phosphate supplement is given to young cattle suffering from a lack of this element, there is an immediate stimulation of the appetite and a more efficient utilization of the food consumed. Milking cows fed ample amounts of nutrients except phosphorus have a rela-

tively short milking period and consequently a low total production. This is highly inefficient because of the relatively large food intake for the year in comparison with returns in the form of milk, the animal having to be maintained dry for a relatively large part of the year. The same cows fed sufficient phosphorus produce much more milk in a more extended lactation period with resulting greatly reduced cost per unit of milk. Growing heifers, either dry or in milk, make much more economical gains in live weight when receiving adequate amounts of phosphorus than animals consuming rations deficient in this element but containing the same amount of total digestible nutrients. The experimental data presented do not explain these results, except to indicate a much more efficient utilization of food when the phosphorus supply is adequate.

14. The annual milk production of cows fed adequate amounts of phosphorus prior to and during lactation is shown to be much greater than when the same animals go through a lactation period with low phosphorus reserves and an inadequate phosphorus supply. The increased milk production in the former instance obtained in the experiments reported ranged from 50 to 146 per cent. Experimental proof is presented that these differences were due solely to the amount of phosphorus ingested. Some of the cows maintained a satisfactory milk production when their ration was limited practically to prairie hay and oats, provided the phosphorus intake was adequate. No evidence is now available, however, that a phosphate supplement will have any effect whatever on the level of milk production when crops fed are produced on soil well supplied with phosphorus and the forage is consequently not abnormally low in this element.

15. Additional experimental data are presented on the relation of magnesium sulfate ingestion to phosphorus deficiency in cattle. Long-time feeding experiments, in which magnesium sulfate was fed as Epsom salt in amounts comparable to the average ingestion of this compound from well water in certain of the phosphorus-deficient regions of Minnesota, do not indicate that the magnesium sulfate ingestion is an important factor in producing phosphorus deficiency. This conclusion is substantiated by balance trials, blood composition, and a study of the bones of the cows fed the Epsom salt for 14 months. No study was made, however, of the effects of extremely "alkali" water such as occurs in isolated areas.

16. A brief survey is given in this bulletin of the present status of our knowledge regarding the effect of phosphorus deficiency on reproduction in dairy cattle. It is evident that the percentage calf crop is considerably reduced as the result of this condition. A more in-

tensive experimental study of this problem is now under way at this Station.

17. The practical aspects of the prevention and treatment of phosphorus deficiency are considered in some detail. The problem is discussed from the following viewpoints: (1) the amount of phosphorus provided by the common forms of dairy feeds available in Minnesota, (2) the increase of forage phosphorus in the phosphorus-deficient areas by means of phosphate fertilization of the soil, (3) the use of mineral supplements and the most economical forms to employ.

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APPENDIX

METHODS OF CHEMICAL ANALYSIS

The following methods of chemical analysis have been employed in these investigations:

Large samples of feed are ground for analysis in a hammer mill and smaller samples in a Wiley mill.

The gross nutrients in the feeds, i.e., dry matter, protein (N × 6.25), ether extract, ash, and nitrogen-free extract, are determined by the official methods of the A. O. A. C., and the results calculated to the moisture-free basis.

The final samples of excreta collected in the balance trials, and preserved with chloroform are usually sampled for analysis without drying, altho the analytical sample is dried on the steam bath before ashing. In a few cases, however, the samples were dried at reduced pressure, the residue was ground and the analyses were made of the dried maturial. The samples of feed consumed and feed refused in the balance trials, to be analyzed for calcium, phosphorus, and magnesium, are usually analyzed in the air-dry state as received from the barn, as the moisture content of the air-dry feed does not affect the calculation of the actual intake of mineral elements.

Prior to 1925, the methods of analysis of feeds for calcium and phosphorus were those recommended by the A. O. A. C. Between 1925 and 1929, the mineral elements in the feeds and in excreta samples (from balance trials) were determined by ashing the sample in a silica dish, taking up the ash in concentrated HCl (1 cc. for each gram of original dry sample), diluting the ash to 100 cc. with water and removing aliquots of this filtered solution for calcium and phosphorus. Calcium was determined by the method described by Kramer and Howland (47), at the same time making use of the washing and draining procedure of Clark and Collip (48). Phosphorus was determined by Briggs' (49) blood phosphate method. Beginning in 1929, the procedures were modified as described by Morris, Nelson, and Palmer (50). At that time, the Fiske-Subbarow (51) method for phosphate supplanted the Briggs method. The estimation of magnesium was included in this procedure. This required a more careful adjustment of the reaction of the solution for the calcium precipitation (pH 5.8 instead of the pH 6.2 recommended by Kramer and Howland), but the precipitation procedure for the magnesium was carried out by the Kramer and Howland method and magnesium estimated from the phosphorus content of the precipitate, using the Fiske-Subbarow method.

Blood analyses are made on plasma prepared from jugular venous blood. The blood is drawn into saturated sodium citrate solution (1 cc. per 100 cc. blood), quickly mixed, and the plasma centrifuged out immediately. Most of blood analyses reported in this bulletin represent a composite of equal parts of plasma prepared on three consecutive days. Total plasma calcium and inorganic phosphates are the usual determinations. Calcium is determined by the Clark-Collip (48) modification of the Kramer-Tisdall procedure. The Briggs (49) modification of the Bell-Doisy colorimetric method for blood phosphate was employed up to 1929. Since that time, the Fiske-Subbarow (51) procedure has been employed because of its greater convenience.

In the study of the bones, the femur, humerus, sixth and eleventh ribs from the right side of the carcass are removed as soon as possible after the animal is slaughtered. The bones with the adhering flesh are frozen immediately. When this is done the flesh is scraped from the bone and longitudinal sections of the femur and humerus are sawed. These sections and the ribs are then broken in an iron mortar and weighed into tared aluminum dishes. The pulverizing of these sections is greatly facilitated by first freezing them with liquid air. The type of saw used and the character of the bone samples are shown in Figure 8. The longitudinal section shown is of uniform thickness when

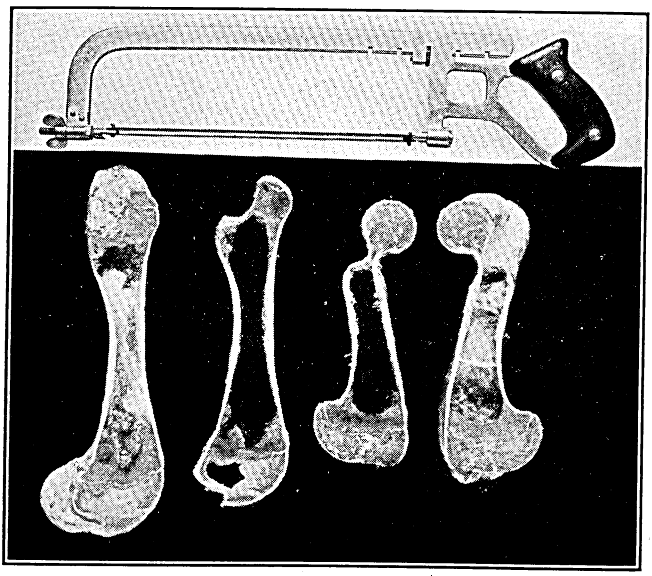

Fig. 8. The Double-Bitted Hack-Saw Used to Saw the Longitudinal Sections for Analyses; Also a Femur and Humerus Together with the Section Obtained from Each by Means of the Saw

prepared with the modified hack saw. It is obtained most easily from the frozen bone. This section more nearly represents proportionately the different parts of the bone than the cross-section usually made. No other equally representative sample of a large bone can be secured so easily or prepared for analysis with less labor.

All samples are dried to constant weight at 100° C. When dried, they are transferred to extraction thimbles and extracted with hot 95 per cent alcohol followed by ether until they are extract-free. The percentage of alcohol-ether extract is calculated from the difference between the dry and the dry-extracted weights. The sample is then reduced in an iron mortar until it will pass a 20-mesh sieve. Ash determinations are made on gram aliquots of this bone powder.

The mineral constituents of the dry extracted bone are determined by the method of Kramer and Howland (47) for unashed bone, except for two modifications. One modification is the precipitation, washing, and titration of the calcium in 75 cc. pointed centrifuge tubes as for blood calcium, but using the quantities of sample and reagents recommended by Kramer and Howland. The other is the determination of the carbonate by absorption in standard alkali, using a set-up very similar to that employed in the Geissler method. The details of the analysis are described by Neal and Palmer (12).

The analysis of water samples for calcium and magnesium are by the official methods of the A. O. A. C., employing the concentrate of sufficient amount of water, evaporated in a platinum dish. Sulfates are determined directly by a turbimetric method against a standard 0.1 per cent solution of potassium sulfate. Graduated cylinders of 50 cc. capacity are used. In the standard tube are placed 10 cc. standard sulfate solution, 20 cc. distilled water, and 5 cc. 10 per cent solution of barium chloride. In the other tube are placed 30 cc. of the unknown water and 5 cc. of the barium chloride. The concentration of sulfates in the unknown is estimated from the depth of the known turbid barium sulfate solution required to match the turbidity of a given depth of the unknown when viewed against a standard light source. The estimation is probably correct within 5 to 10 per cent.

Table 1

E 75, Dec. 6 to 15, 1926 (10 Days); Non-Lactating; Weight 801 Pounds; Blood Plasma, Total Ca 12.92, Inorganic P 1.75 mgm. per 100 cc.; Low P Hay, $CaCO_3$ Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	59.02	0.454	0.073	267.95	43.08
Oats	11.35	0.135	0.252	15.32	28.60
$CaCO_3$	1.00	....	....	400.00	....
Water	99.97	0.0049	....	4.90	....
Gross intake	....	....	....	*688.17*	*71.68*
Refused feed	10.58	0.039	0.056	32.69	5.92
Net intake	....	....	....	*655.48*	*65.76*
Excreta	125.48	0.539	0.050	703.23	65.23
Balance	....	....	....	*−47.75*	*+0.53*
Balance per day	....	....	....	*−4.75*	*+0.053*

Table 2

E 75, Jan. 13 to 22, 1927 (10 Days); Non-Lactating; Weight 814 Pounds; Blood Plasma, Total Ca 12.78, Inorganic P 6.80 mgm in 100 cc.; Low P Hay, $Ca_3(PO_4)_2$ Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	45.40	0.470	0.075	213.38	34.05
Oats	13.62	0.102	0.297	13.89	40.45
$Ca_3(PO_4)_2$	1.00	....	....	386.94	202.00
Water	98.57	0.0049	....	4.83	....
Gross intake	....	....	....	*619.04*	*276.50*
Refused feed	0.79	0.470	0.075	3.71	0.59
Net intake	....	....	....	*615.33*	*275.91*
Excreta	112.14	0.421	0.211	493.07	247.12
Balance	....	....	....	*+122.26*	*+28.79*
Balance per day	....	...	....	*+12.23*	*+2.88*

Table 3

E 93, Dec. 30, 1926 to Jan. 8, 1927 (10 Days); Non-Lactating; Weight 760 Pounds; Blood Plasma, Total Ca 13.12, Inorganic P 1.58 mgm. per 100 cc.; Low P Hay $CaCO_3$ Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	49.94	0.348	0.067	173.79	33.46
Oats	9.08	0.100	0.262	9.08	23.79
$CaCO_3$	1.00	....	....	400.00	....
Water	84.47	0.0049	....	4.14	....
Gross intake	....	....	....	*587.01*	*57.25*
Refused feed	0.14	0.348	0.067	0.48	0.08
Net intake	....	....	....	*586.53*	*57.17*
Excreta	144.37	0.455	0.042	679.63	62.73
Balance	....	....	....	*−39.10*	*−5.56*
Balance per day	....	....	....	*−9.31*	*−0.56*

Table 4

E 93, Feb. 2 to 11, 1927 (10 Days); Non-Lactating; Weight 760 Pounds; Blood Plasma, Total Ca 12.13, Inorganic P 4.94 mgm. per 100 cc.; Low P Hay, $Ca_3(PO_4)_2$ Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	54.48	0.315	0.067	171.61	36.50
Oats	9.08	0.114	0.291	10.35	26.42
$Ca_3(PO_4)_2$	1.00	....	....	386.94	202.00
Water	108.96	0.0049	....	5.34	....
Gross intake	....	....	....	*574.24*	*264.92*
Refused feed	0.0	....	....	....	....
Net intake	....	....	....	*574.24*	*264.92*
Excreta	137.38	0.289	0.112	426.82	165.41
Balance	....	....	....	*+147.42*	*+99.51*
Balance per day	....	....	....	*+14.74*	*+9.95*

Table 5

E 94, Dec. 6 to 15, 1926 (10 Days); Non-Lactating; Weight 775 Pounds; Blood Plasma, Total Ca 13.68, Inorganic P 0.77 mgm. per 100 cc.; Low P Hay, No Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	54.48	0.454	0.073	247.34	39.77
Oats	11.35	0.135	0.252	15.32	28.60
Water	135.06	0.0049	....	6.62	....
Gross intake	....	....	....	*269.28*	*68.37*
Refused feed	4.45	0.479	0.056	13.75	2.49
Net intake	....	....	....	*255.63*	*65.88*
Excreta	163.75	0.133	0.032	224.44	54.00
Balance	....	....	....	*+31.09*	*+11.88*
Balance per day	....	....	....	*+3.11*	*+1.19*

Table 6

E 94, Jan. 13 to 22, 1927 (10 Days); Non-Lactating; Weight 776 Pounds; Blood Plasma, Total Ca 11.54, Inorganic P 6.06 mgm. per 100 cc.; Low P Hay, $NaH_2PO_4 \cdot H_2O$ Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	49.94	0.470	0.075	234.72	37.45
Oats	13.62	0.102	0.297	13.89	40.45
$NaH_2PO_4 \cdot H_2O$	1.00	....	....	....	224.96
Water	163.55	0.0049	....	8.01	....
Gross intake	....	....	....	*256.62*	*302.86*
Refused feed	0.0	....	....	....	....
Net intake	....	....	....	*256.62*	*302.86*
Excreta	164.48	0.059	0.091	102.95	158.78
Balance	....	....	....	*+153.67*	*+144.08*
Balance per day	....	....	....	*+15.37*	*+14.41*

Table 7

E 92, Mar. 17 to 26, 1927 (10 Days); Lactating; Weight 871 Pounds; Blood Plasma, Total Ca 10.92, Inorganic P 5.08 mgm. per 100 cc.; Low P Hay, $NaH_2PO_4 \cdot H_2O$ Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	54.48	0.335	0.062	182.51	33.77
Oats	63.56	0.110	0.285	69.91	181.14
$NaH_2PO_4 \cdot H_2O$	1.00	....	....	....	224.96
Water	284.97	0.0049	....	13.96	....
Gross intake	....	....	....	*266.38*	*439.87*
Refused feed	0.23	0.335	0.062	0.77	0.14
Net intake	....	....	....	*265.61*	*439.73*
Excreta	235.4	0.067	0.111	164.41	272.39
Total assimilated	....	....	....	*101.20*	*167.34*
Milk	119.4	0.118	0.095	140.89	113.43
Balance	....	....	....	*−39.69*	*+53.91*
Balance per day	....	....	....	*−3.97*	*+5.39*

Table 8

E 92, May 10 to 20, 1927 (10 Days); Lactating; Weight 973 Pounds; Blood Plasma, Total Ca 11.02, Inorganic P 2.04 mgm. per 100 cc.; Low P Hay, No Mineral Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	68.10	0.272	0.052	185.23	35.41
Oats	63.56	0.106	0.227	67.37	152.93
Water	335.05	0.0049	....	16.42	....
Gross intake	....	....	....	*269.02*	*188.34*
Refused feed	3.74	0.481	0.039	17.90	1.46
Net intake	....	....	....	*251.12*	*186.88*
Excreta	270.84	0.090	0.044	252.76	123.57
Total assimilated	....	....	....	*−1.64*	*+63.31*
Milk	103.65	0.108	0.100	111.94	103.65
Balance	....	....	....	*−113.58*	*−40.34*
Balance per day	....	....	....	*−11.36*	*−4.03*

Table 9

E 74, May 11 to 20, 1927 (10 Days); Lactating; Weight 1,009 Pounds; Blood Plasma, Total Ca 11.21, Inorganic P 3.72 mgm. per 100 cc.; Low P Hay, $NaH_2PO_4 \cdot H_2O$ Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	63.56	0.272	0.052	172.88	33.05
Oats	54.48	0.106	0.227	57.75	123.67
$NaH_2PO_4 \cdot H_2O$	1.00	....	....	....	224.96
Water	322.34	0.0049	....	15.79	....
Gross intake	....	....	....	*246.42*	*381.68*
Refused feed	0.0	....	....	....	....
Net intake	....	....	....	*246.42*	*381.68*
Excreta	257.43	0.086	0.106	221.39	272.87
Total assimilated	....	....	....	*25.03*	*108.81*
Milk	88.8	0.127	0.101	95.90	88.80
Balance	....	....	....	*−70.87*	*+20.01*
Balance per day	....	....	....	*−7.09*	*+2.00*

Table 10

E 74, July 6 to 16, 1927 (10 Days); Lactating; Weight 1,002 Pounds; Blood Plasma, Total Ca 11.12, Inorganic P 3.82 mgm. per 100 cc.; Low P Hay, No Supplement

	Weight	Analysis		Mineral content	
		Ca	P	Ca	P
	kg.	per cent	per cent	gm.	gm.
Prairie hay	68.10	0.303	0.071	206.34	48.35
Oats	54.48	0.119	0.242	64.28	130.20
Water	377.73	0.0049	....	18.88	....
Gross intake	....	....	....	*289.50*	*178.55*
Refused feed	2.95	0.260	0.058	11.82	1.42
Net intake	....	....	....	*277.68*	*177.13*
Excreta	288.81	0.074	0.046	274.37	118.41
Total assimilated	....	....	....	*3.31*	*58.72*
Milk	67.55	0.148	0.102	85.11	66.20
Balance	....	....	....	*−81.80*	*−7.48*
Balance per day	....	....	....	*−8.18*	*−0.75*

Table 11

E 121. Feed Intake and Blood Analyses 180 Days Following Record in Table 27

During period when extreme symptoms of phosphorus deficiency developed.

Period No.	Thirty day period ending	Weight end of period	Daily ration: Prairie hay	Daily ration: Corn	Daily ration: Oats	Daily ration: Corn gluten meal	Daily ration: Mo-lasses	Daily ration: Dry beet pulp	Daily ration: Epsom salt	Nutrient intake per day: Digest-ible protein	Nutrient intake per day: Total digestible nutrients	Mineral intake per day: Ca	Mineral intake per day: P	Mineral intake per day: Mg	Ca and P in 100 cc.* blood plasma: Ca	Ca and P in 100 cc.* blood plasma: Inorganic P
		lb.	lb.	lb.	lb.	lb.	lb.	lb.	gm.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
1	12/7/29	874	12.0	0.86	0.43	1.78	2.0	..	150	1.07	8.80	40.80	7.40	30	11.19	2.53
2	1/6/30	897	10.6	0.86	0.43	1.78	2.0	2.1	150	1.13	9.53	38.06	7.97	32	9.55	2.01
3	2/5/30	905	10.3	0.40	0.80	1.60	2.0	3.0	35	1.13	9.89	47.14	8.19	21	9.85	1.65
4	3/7/30	910	10.6	0.43	0.86	1.78	2.0	3.0	...	1.19	10.25	47.83	9.02	18	10.41	1.75
5	4/6/30	870	7.4	0.42	0.84	1.68	1.9	2.9	...	1.09	8.77	38.76	8.04	15	11.28	2.23
6	5/6/30	862	6.5	0.41	0.83	1.66	1.9	2.9	...	1.04	8.21	35.90	7.92	14	11.70	2.03
7	6/5/30	844	5.4	0.35	0.69	1.38	3.1	3.1	...	0.93	8.19	38.66	7.35	14	11.23	1.92
8	7/5/30	842	5.3	0.22	0.44	0.88	1.7	2.1	...	0.66	5.92	28.91	4.70	10	11.69	2.76

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days during the 30-day period.

Table 12

E 147. Feed Intake and Blood Analyses—Severe, But Typical Phosphorus Deficiency Symptoms Developed

Age at beginning—276 days; weight 414 pounds.

Period No.	Thirty day period ending	Weight end of period	Daily ration: Prairie hay	Daily ration: Alfalfa hay	Daily ration: Beet pulp	Daily ration: Oats	Daily ration: Corn	Daily ration: Corn gluten meal	Daily ration: Starch	Daily ration: Molasses	Nutrient intake per day: Digestible proteins	Nutrient intake per day: Total digestible nutrients	Mineral intake per day: Ca	Mineral intake per day: P	Ca and P in 100* cc. blood plasma: Ca	Ca and P in 100* cc. blood plasma: Inorganic P
		lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	gm.	gm.	mgm.	mgm.
1	1/31/31	472	...	6.27	...	1.86	1.80	0.93	...	...	1.29	6.64	38.03	10.30	11.43	6.94
2	3/2/31	494	4.23	2.07	...	0.73	1.05	1.63	1.27	0.63	1.12	6.82	24.59	6.86	11.05	3.12
3	4/1/31	527	4.80	...	1.58	...	0.08	2.31	2.31	0.16	1.14	7.08	15.75	7.08	10.68	2.20
4	5/1/31	559	3.90	...	2.00	...	0.38	2.50	2.50	...	1.27	7.44	14.21	4.85	11.58	2.24
5	5/31/31	581	3.80	...	2.00	...	0.95	2.05	2.50	...	1.03	7.55	13.54	5.05	11.89	2.65
6	6/30/31	580	3.67	...	1.86	...	0.93	1.86	2.32	...	0.88	7.13	12.65	4.79	11.49	2.67
7	7/30/31	580	4.08	...	1.85	...	1.39	1.85	1.39	...	0.93	6.93	13.52	5.34	10.84	1.93
8	8/29/31	607	3.90	...	0.96	...	1.98	1.68	1.26	...	0.86	6.44	10.85	5.43	10.25	2.42
9	9/28/31	636	3.88	...	0.20	0.36	1.59	2.22	2.49	$CaCO_3$	0.99	7.29	9.04	6.02	10.02	2.65
10	10/28/31	654	3.73	...	0.84	0.26	1.85	2.38	2.22	...	1.13	7.75	10.24	6.33	10.52	2.37
11	11/27/31	677	3.12	...	2.00	0.22	2.50	2.28	1.50	...	1.28	8.15	11.89	6.88	9.65	2.30
12	12/27/31	672	2.76	...	1.66	0.62	2.07	1.66	1.14	9.55†	1.16	7.64	10.97	6.53	10.02	1.68
13	1/26/32	664	2.87	...	1.03	0.51	1.51	1.03	0.53	10.37†	0.90	5.99	17.21	5.30	8.33	2.94

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days during the 30-day period.

† Grams.

Table 13

Analyses of Feedstuffs—Natural Feeds vs. Inorganic Phosphorus Supplements, Air-Dry Basis

Feedstuff	Lot No.	Dry matter	Ash	Crude fiber	Crude protein	Ether extract	N-free extract	Ca	P	Mg
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Prairie hay	28	84.73	7.13	24.98	5.43	3.87	43.32	0.603	0.070	0.232
" "	32	88.66	6.44	28.90	5.01	3.31	45.00	0.494	0.057	0.235
Cottonseed meal	2	90.54	6.32	9.07	42.25	7.75	25.48	0.206	1.295	0.673
Corn gluten meal	9	88.43	1.36	4.98	41.79	2.47	37.72	0.058	0.142	0.050
" " "	18	88.91	1.01	2.40	44.20	3.09	38.21	0.033	0.247	0.025
" " "	20	90.52	1.00	1.70	45.78	2.32	39.72	0.056	0.261	0.031
Corn	15	87.28	1.12	2.63	9.25	3.41	70.46	0.008	0.217	0.131
"	16	83.92	1.38	1.99	8.59	2.23	69.73	0.009	0.320	0.133
"	17	86.13	1.49	2.60	9.91	3.12	69.01	0.021	0.248	0.130
"	18	85.71	1.41	2.74	9.28	2.95	69.33	0.011	0.260	0.122
Oats	17	89.16	3.40	10.52	12.38	3.73	60.38	0.075	0.261	0.114
"	18	87.45	3.42	10.17	12.33	2.51	59.02	0.059	0.317	0.126
Wheat bran	3	85.31	6.60	10.65	15.42	5.09	49.29	0.084	1.429	0.607
Molasses	8	70.57	5.87	....	4.86	1.16	58.68	0.946	0.062	0.237
"	9	67.03	7.98	....	4.39	0.52	54.14	1.022	0.061	0.358

Table 14

Lot Number of Feedstuffs and Periods When Fed
Natural Feeds vs. Inorganic Phosphorus Supplement

Feedstuff	Lot No.	E 118 Period fed from	inclusive to	E 120 Period fed from	inclusive to	E 125 Period fed from	inclusive to	Period fed from	inclusive to
Prairie hay	28	11/21/29	5/8/30	11/21/29	1/12/30	11/21/29	3/8/30		
Cottonseed meal	2	11/21/29	5/8/30	....	....	11/21/29	5/8/30		
Corn gluten meal	9	....	....	11/21/29	1/12/30	....	....		
Corn	15	11/21/29	5/8/30	11/21/29	1/12/30	11/21/29	5/8/30		
Oats	17	11/21/29	5/8/30	11/21/29	1/13/30	11/21/29	5/8/30		
Bran	3	2/25/30	5/8/30	....	....	2/25/30	5/8/30		
Molasses	8	11/21/29	5/8/30	11/21/29	1/12/30	11/21/29	5/8/30		
		E 150		**E 151**		**E 152**		**E 153**	
Prairie hay	28	6/20/30	7/20/30	6/20/30	7/20/30	6/20/30	7/20/30	6/20/30	7/20/30
" "	32	7/21/30	11/13/30	7/21/30	11/13/30	7/21/30	9/11/30	7/21/30	9/11/30
Cottonseed meal	2	....	....	6/20/30	11/13/30	6/20/30	9/11/30	....	....
Corn gluten meal	18	6/20/30	9/3/30	....	....	....	....	6/20/30	9/3/30
" " "	20	9/4/30	11/13/30	....	....	....	....	9/4/30	9/11/30
Corn	16	6/20/30	7/25/30	6/20/30	7/25/30	6/20/30	7/25/30	6/20/30	7/25/30
"	17	7/26/30	9/3/30	7/26/30	9/3/30	7/26/30	9/3/30	7/26/30	9/3/30
"	18	9/4/30	11/13/30	9/4/30	11/13/30	9/4/30	9/11/30	9/4/30	9/11/30
Oats	17	6/20/30	9/3/30	6/20/30	9/3/30	6/20/30	9/3/30	6/20/30	9/3/30
"	18	9/4/30	11/13/30	9/4/30	11/13/30	9/4/30	9/11/30	9/4/30	9/11/30
Molasses	8	6/20/30	8/27/30	6/20/30	8/27/30	6/20/30	8/27/30	6/20/30	8/27/30
"	9	8/28/30	11/13/30	8/28/30	11/13/30	8/28/30	9/11/30	8/28/30	9/11/30

Table 15

Mineral Analyses of Prairie Hay, Air-dry basis
Samples Collected as Part of a Survey of the Prosphorus-Deficient Area.
Each sample represents a different farm.

Sample No.	Ca	P	Mg	Sample No.	Ca	P	Mg
	per cent	per cent	per cent		per cent	per cent	per cent
1	0.450	0.392	0.277	26	0.617	0.153	0.163
2	0.440	0.013	0.271	27	0.493	0.113	0.265
3	0.585	0.013	0.217	28	0.628	0.061	0.295
4	0.210	0.078	....	29	0.400	0.122	0.422
5	0.264	0.083	0.139	30	0.493	0.100	0.211
6	0.364	0.070	0.078	31	0.528	0.135	0.235
7	0.221	0.074	0.066	32	0.293	0.118	0.229
8	0.214	0.061	0.122	33	0.721	0.135	0.193
9	0.493	0.065	0.169	34	0.285	0.131	0.211
10	0.390	0.092	0.241	35	0.293	0.135	0.145
11	0.343	0.122	0.253	36	0.685	0.105	0.404
12	0.571	0.087	0.229	37	0.450	0.109	0.247
13	0.493	0.187	0.332	38	0.450	0.092	0.446
14	0.490	0.170	0.241	39	0.393	0.104	0.253
15	0.290	0.161	0.151	40	0.264	0.126	0.181
16	0.550	0.122	0.290	41	0.543	0.100	0.489
17	0.329	0.065	0.200	42	0.428	0.100	0.217
18	0.290	0.046	0.169	43	0.478	0.148	0.302
19	0.221	0.100	0.169	44	0.643	0.065	0.205
20	0.467	0.092	0.235	45	0.462	0.131	0.350
21	0.493	0.083	....	46	0.779	0.066	....
22	0.379	0.092	....	47	0.272	0.083	....
23	0.686	0.100	....	48	0.665	0.131	....
24	0.546	0.079	....	49	0.436	0.092	....
25	0.350	0.066	....	50	0.472	0.105	....
				51	0.193	0.114	....
Average					*0.440*	*0.106*	*0.233*

Table 16

Analyses of Prairie Hay Used in Experimental Work, Air-Dry Basis

Lot No.	Moisture	Crude protein	Crude fiber	N-free extract	Ether extract	Ash	Ca	P	Mg
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
			Prairie hay produced on low-phosphorus soil						
1	8.82	5.48	27.03	49.46	2.63	6.56	0.572	0.087	....
2	9.44	5.59	28.25	47.52	2.26	6.95	0.515	0.092	....
3	9.33	5.09	30.14	46.33	1.75	7.16	0.522	0.109	....
17	8.62	8.18	30.03	44.20	2.39	6.68	0.329	0.074	....
18	8.17	7.41	29.70	45.24	1.93	7.55	0.374	0.076	....
19	9.29	6.50	28.24	47.70	1.90	6.37	0.415	0.138	....
21	6.80	5.81	30.06	49.53	2.51	5.30	0.426	0.087	....
22	7.74	6.25	30.56	45.01	2.21	8.23	0.260	0.061	....
23	7.61	5.35	31.16	46.06	2.46	7.36	0.372	0.034	....
24	7.85	6.72	30.04	46.29	2.29	6.81	0.352	0.062	....
25	14.78	5.26	25.68	43.77	3.38	7.14	0.301	0.069	....
26	15.16	5.80	26.00	42.99	3.42	6.63	0.307	0.065	....
27	14.04	6.41	24.19	44.42	3.24	7.69	0.260	0.067	....
28	15.27	5.43	24.98	43.32	3.87	7.13	0.603	0.070	....
29	11.57	5.62	28.39	45.60	4.23	4.59	0.549	0.068	0.232
32	11.34	5.01	28.90	45.00	3.31	6.44	0.494	0.057	0.235
34	17.58	5.01	25.18	43.43	2.68	6.12	0.466	0.061	0.304
36	14.96	5.37	27.42	43.19	3.34	5.72	0.419	0.046	0.250
44	13.81	6.00	27.01	42.21	3.24	7.73	0.485	0.061	0.337
45	12.14	5.85	23.52	47.89	2.96	7.64	0.401	0.071	0.262
46	16.04	4.63	26.59	41.98	2.89	7.87	0.525	0.046	0.302
Av.	*11.44*	*5.85*	*27.76*	*45.30*	*2.80*	*6.84*	*0.426*	*0.071*	*0.270*
		Prairie hay purchased on Minneapolis market—source unknown							
33	9.91	4.76	26.20	47.09	3.70	8.34	0.607	0.078	0.142
35	13.19	7.30	26.56	41.84	3.67	7.44	0.485	0.085	0.256
43	11.76	7.30	26.99	43.53	3.31	7.11	0.518	0.088	0.382
Av.	*11.62*	*6.45*	*26.58*	*44.15*	*3.56*	*7.63*	*0.531*	*0.084*	*0.260*

Table 17

Mineral Analyses of Timothy Hay Used in Experimental Work, Air-Dry Basis

Lots 4 to 16 Minneapolis Market, 18 to 31 High Acid Soil in Wisconsin

Lot No.	Moisture	Crude protein	Crude fiber	N-free extract	Ether extract	Ash	Ca	P	Mg
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
4	6.40	5.72	29.84	50.15	2.23	5.75	0.179	0.240	....
5	7.41	7.50	30.69	44.33	2.39	7.68	0.296	0.101	....
8	5.27	5.08	27.75	53.70	3.51	4.69	0.185	0.137	....
9	11.26	5.73	27.88	46.85	3.39	4.89	0.234	0.093	0.107
10	10.80	5.78	28.24	46.74	2.68	5.91	0.409	0.181	0.139
11	9.73	6.07	29.19	46.41	3.04	4.69	0.298	0.144	0.123
12	9.22	4.81	32.95	44.79	1.89	3.66	0.225	0.119	0.116
13	10.41	9.54	33.73	37.59	3.30	4.81	0.473	0.115	0.179
14	9.13	5.33	28.42	47.65	3.84	4.57	0.400	0.160	0.160
15	9.59	5.32	27.82	50.44	2.85	3.72	0.321	0.166	0.145
32	8.51	8.47	28.32	46.01	2.46	6.23	0.414	0.099	0.233
16	9.73	6.76	32.03	43.05	3.11	4.61	0.403	0.176	0.163
Av.	*8.95*	*6.34*	*29.73*	*46.48*	*2.89*	*5.10*	*0.319*	*0.144*	*0.151*
18	9.12	5.44	29.91	47.49	2.93	3.66	0.299	0.160	0.109
19	8.46	5.59	31.39	46.24	2.53	3.93	0.293	0.156	0.113
20	11.25	5.23	32.90	44.03	3.35	3.67	0.287	0.125	0.107
21	9.45	5.02	33.19	44.36	3.52	4.15	0.284	0.136	0.114
22	14.11	5.46	30.72	43.41	3.62	4.01	0.330	0.133	0.138
23	12.14	6.32	32.87	41.86	2.23	4.58	0.215	0.168	0.098
24	11.26	6.60	29.62	45.03	3.24	4.25	0.310	0.144	0.152
25	12.57	8.17	28.84	43.10	3.00	4.32	0.294	0.135	0.131
26	6.03	4.73	34.10	47.29	3.81	4.04	0.235	0.116	0.111
34	12.01	5.58	29.16	46.13	2.56	4.56	0.285	0.128	0.134
35	12.05	6.60	30.01	43.87	2.58	4.89	0.335	0.154	0.159
Av.	*10.76*	*5.88*	*31.15*	*44.80*	*3.03*	*4.18*	*0.288*	*0.141*	*0.124*

Table 18

Mineral Analyses of Timothy Hay, Air-Dry Basis

Samples collected as part of a survey of phosphorus-deficient area. Each sample represents a different farm.

Sample No.	Ca	P	Mg
	per cent	per cent	per cent
1	0.521	0.078	0.241
2	0.406	0.113	0.235
3	0.385	0.109	0.180
4	0.257	0.126	0.120
5	0.407	0.135	0.211
Average	*0.395*	*0.112*	*0.197*

Table 19

Mineral Analyses of Alfalfa Hay, Air-dry Basis

Samples collected as part of a survey of phosphorus deficient area. Each sample represents a different farm.

Sample No.	Ca	P	Mg	Sample No.	Ca	P	Mg
	per cent	per cent	per cent		per cent	per cent	per cent
1	1.606	0.113	0.422	13	2.263	0.209	0.464
2	1.785	0.087	0.639	14	1.849	0.235	0.476
3	2.328	0.156	0.434	15	1.335	0.344	0.645
4	1.871	0.170	0.380	16	1.628	0.235	0.349
5	2.201	0.191	0.349	17	1.606	0.283	....
6	1.777	0.187	0.307	18	1.535	0.244	0.319
7	1.521	0.170	0.325	19	2.050	0.191	0.295
8	1.606	0.204	0.464	20	1.871	0.279	0.343
9	2.234	0.239	0.464	21	2.077	0.318	0.446
10	2.492	0.252	0.343	22	1.042	0.252	0.331
11	1.535	0.183	0.494	23	2.056	0.156	0.428
12	1.564	0.248	0.422	24	1.308	0.153	....
Av for 24 samples					*1.81*	*0.212*	*0.397*

Table 20

Analyses of Alfalfa Hay Used in Experimental Work,* Air-Dry Basis

Lot No.	Moisture	Crude protein	Crude fiber	N-free extract	Ether extract	Ash	Ca	P	Mg
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	5.52	16.79	29.21	38.12	2.83	7.52	1.608	0.284	....
2	7.08	16.12	25.27	40.58	2.84	8.10	1.380	0.214	....
3	9.63	13.78	34.71	33.87	2.30	5.70	1.465	0.188	....
6	7.06	15.40	33.29	36.65	2.11	5.49	1.150	0.186	....
10	10.63	15.49	28.70	34.34	2.61	8.21	1.613	0.234	....
11	10.27	14.84	29.53	35.54	2.25	7.56	1.432	0.188	....
12	7.15	11.94	36.63	33.39	2.20	8.71	1.597	0.232	....
13	7.83	16.92	25.01	39.76	2.10	8.38	0.898	0.155	....
14	9.15	15.50	27.55	37.39	3.45	6.96	0.863	0.215	....
15	8.78	16.00	29.00	35.57	2.73	7.93	0.862	0.215	....
16	8.66	15.87	26.27	40.03	2.78	6.39	1.720	0.283	....
21	6.00	13.47	31.89	38.82	3.37	6.44	1.108	0.128	....
22	6.64	16.15	29.02	37.66	3.92	6.60	1.369	0.250	....
23	9.38	17.43	35.02	29.12	3.14	5.90	1.438	0.227	....
24	8.99	15.31	33.06	33.13	3.16	5.89	1.494	0.230	0.304
25	9.90	13.22	33.56	37.63	2.15	4.50	1.202	0.225	0.304
26	13.62	17.45	20.56	37.02	3.71	5.99	1.889	0.260	0.335
27	15.69	12.73	24.08	36.10	3.77	7.63	1.639	0.145	0.438
28	13.52	13.21	29.09	35.41	2.64	6.03	1.267	0.161	0.199
31	10.15	15.30	23.14	41.11	2.79	7.51	1.970	0.164	0.397
32	9.49	13.59	31.81	35.00	2.60	7.51	1.107	0.231	0.224
Av.	*9.30*	*15.07*	*29.35*	*36.49*	*2.83*	*6.90*	*1.389*	*0.210*	*0.314*

* All samples purchased on Minneapolis market. Possibly all grown in Minnesota except Lot 32, known to have come from Kansas.

Table 21

Nutrient and Mineral Intake in Relation to Gain in Live Weight of Growing Cattle

Group 1, phosphorus-deficient ration. Group 2, phosphorus-deficient ration plus Epsom salt. Group 3, phosphorus-deficient ration plus Epsom salt plus sodium phosphate

No. of animal	Period	Weight at start	Daily gain	Minerals received daily		Total digestible nutrients received daily	Total digestible nutrients per lb. of gain	Total digestible nutrients above maintenance*	
				Ca	P			Received daily	Per lb. of gain
	days	lb.	lb.	gm.	gm.	lb.	lb.	lb.	lb.
Group 1									
E 120	174	711	0.966	26.98	8.42	9.60	9.94	3.29	3.41
E 123	174	467	1.092	23.48	7.44	8.34	7.64	3.57	3.27
E 125	174	460	1.069	21.10	6.78	7.46	6.98	2.74	2.56
Av.	174	546	1.042	23.85	7.55	8.47	8.13	3.20	3.08
Group 2									
E 118	174	841	1.155	26.82	8.47	9.66	8.36	2.60	2.25
E 121	174	610	1.115	25.68	8.06	9.16	8.22	3.46	3.10
E 122	174	632	1.465	25.71	8.09	9.15	6.25	3.31	2.26
Av.	174	694	1.245	26.07	8.21	9.32	7.49	3.12	2.54
Group 3									
E 124	174	750	1.230	27.23	16.82	9.74	7.92	3.20	2.60
E 126	174	685	1.149	24.81	16.08	8.83	7.68	2.67	2.32
E 127	174	650	1.379	25.20	16.13	8.89	6.45	2.94	2.13
Av.	174	695	1.252	25.78	16.34	9.15	7.31	2.94	2.35
Group 1									
E 120	240	879	0.504	29.60	7.29	8.70	17.26	1.43	2.84
E 123	240	657	0.371	30.22	7.33	8.86	23.88	2.87	7.74
E 125	240	646	0.242	29.61	7.17	8.53	35.25	2.61	10.79
Av.	240	727	0.372	29.81	7.26	8.70	23.39	2.30	7.12
Group 2									
E 118	240	1,042	0.367	36.11	8.22	10.16	27.68	2.01	5.48
E 121	240	804	0.258	29.96	7.36	8.81	34.15	1.96	7.60
E 122	240	887	0.583	34.68	8.07	9.87	16.93	2.55	4.37
Av.	240	911	0.403	33.58	7.88	9.61	23.85	2.17	5.82
Group 3									
E 124	240	964	0.979	39.05	22.46	10.82	11.05	3.09	3.16
E 126	240	885	0.925	37.81	21.46	10.54	11.39	3.23	3.49
E 127	240	890	0.950	37.10	21.74	10.47	11.02	3.14	3.31
Av.	240	913	0.951	37.99	21.89	10.61	11.16	3.15	3.32

* The maintenance requirements in Tables 21 and 22 were calculated from the Haecker figures of 7.925 pounds total digestible nutrients for an animal weighing 1,000 pounds, according to the formula: Maintenance $= 7.925 \left(\frac{\text{Desired weight}}{1{,}000}\right)^{2/3}$

Table 22

Effect of Phosphate Intake on Nutrients Required per Pound of Gain in Weight

No. of animal	Length of period	Weight at start	Daily gain	F. C. M.*	Nutrients received daily: Crude protein	Nutrients received daily: Total digestible nutrients	Minerals in ration daily: Ca	Minerals in ration daily: P	Estimated phosphorus required daily	Phosphorus received above milk and maintenance requirements	Total digestible nutrients required per pound of gain
	days	lb.	lb.	lb.	lb.	lb.	gm.	gm.	gm.	gm.	lb.
E 75	240	810	0.621	....	0.71	7.61	55.74	25.77	8.10	17.67	1.16
E 60	150	472	0.900	2.71	0.71	6.82	36.99	18.92	7.43	11.49	1.22
E 93	270	740	1.089	....	0.70	7.97	57.55	26.82	7.40	19.42	1.37
E 59	120	928	1.340	3.49	1.14	10.61	26.29	34.76	12.77	21.99	1.40
E 94	150	701	0.733	19.82	1.64	14.05	24.57	39.22	26.83	12.39	1.42
E 74	235	714	1.310	....	0.73	8.29	28.43	23.03	7.14	15.89	1.50
E 62	330	463	0.400	5.10	0.73	7.15	18.18	26.47	9.73	16.74	1.67
E 92	210	733	1.320	10.31	1.28	12.35	32.43	34.69	17.64	17.05	1.82
E 58	360	534	0.660	3.85	0.81	7.90	18.80	33.20	9.19	24.01	2.08
E 75	330	754	0.685	13.59	1.46	12.85	34.23	35.94	21.13	14.81	2.41
E 58	180	682	0.861	17.24	1.48	14.28	35.76	39.56	24.06	15.50	2.62
E 91	120	602	0.483	....	0.63	7.52	27.38	6.16	6.02	0.14	3.87
E 93	180	624	0.322	12.58	1.01	11.60	66.65	14.27	18.82	−3.55	4.72
E 73	355	695	0.448	....	0.76	8.48	29.05	7.62	6.95	0.67	5.04
E 91	300	543	0.683	13.18	1.59	13.62	23.41	17.37	18.61	−1.24	5.65
E 36	330	627	0.270	4.04	0.72	8.72	93.00	11.82	10.31	1.51	5.70
E 59	210	878	0.238	6.92	1.12	11.48	71.51	15.75	15.70	0.05	7.77
E 33	285	656	0.147	7.23	0.80	10.00	40.14	12.93	11.79	1.14	10.61
E 75	265	760	0.087	11.67	1.29	12.07	61.40	15.07	19.27	−4.20	17.13
E 33	365	889	0.066	20.89	1.88	16.27	35.09	22.30	29.78	−7.48	27.58

* Milk yield corrected to 4 per cent fat using the formula F. C. M. = 0.4M + 15F.

Table 23

E 120, Group 1. Basal Ration, Low Phosphorus

Thirty-day period ending	Weight end of period	Daily ration			Nutrient intake per day		Mineral intake per day			Ca and P in 100 cc. blood plasma†	
		Prairie hay	Grain mixture	Molasses	Digestible proteins	Total digestible nutrients	Ca	P	Mg	Ca	Inorganic P
	lb.	lb.	lb.	lb.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
10/13/28*........	741	13.25	3.83	2.00	1.07	9.26	24.28	8.64	17.12	....	....
11/12/28.........	768	14.60	3.48	1.80	1.04	9.52	25.51	8.65	17.81	10.76	5.67
12/12/28.........	801	14.73	3.50	2.00	1.07	9.64	26.42	8.67	17.63	....	....
1/11/29.........	810	13.32	3.95	2.00	1.14	10.11	27.52	8.44	14.27	10.86	5.25
2/10/29.........	863	13.53	3.05	2.00	0.98	9.48	27.56	7.69	14.07	10.10	5.67
3/12/29.........	879	15.29	2.08	2.00	0.88	9.51	30.05	8.48	15.66	9.59	5.65
4/11/29.........	875	15.04	2.38	2.00	0.97	9.62	27.64	8.99	13.62	10.81	4.11
5/11/29.........	888	12.58	2.87	2.00	1.07	8.79	23.26	6.87	10.41	10.66	4.17
6/10/29.........	902	11.13	3.00	2.00	1.09	9.26	21.51	6.49	9.60	11.80	3.61
7/10/29.........	938	11.25	3.17	2.00	1.13	8.55	23.53	7.54	10.95	10.58	3.78
8/ 9/29.........	953	10.84	3.02	1.53	1.04	8.30	33.32	7.13	14.73	10.51	3.57
9/ 8/29.........	948	10.14	3.00	2.00	1.02	8.19	33.23	6.83	14.36	10.50	4.29
10/ 8/29.........	963	11.44	3.00	2.00	1.06	8.81	36.44	7.14	15.75	11.90	3.40
11/ 7/29.........	1,000	12.02	3.00	2.00	1.07	9.08	37.89	7.32	16.37	9.45	3.21

* Twenty-four days in this period.

† The Ca and P figures represent a composite sample made from blood samples drawn on three successive days in the 30-day period.

Table 24

E 123, Group 1. Basal Ration, Low Phosphorus

Thirty-day period ending	Weight end of period	Daily ration			Nutrient intake per day		Mineral intake per day			Ca and P in 100 cc. blood plasma*	
		Prairie hay	Grain mixture	Molasses	Digestible proteins	Total digestible nutrients	Ca	P	Mg	Ca	Inorganic P
	lb.	lb.	lb.	lb.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
10/13/28.........	510	11.46	3.25	2.00	0.92	8.06	21.89	7.53	15.41	....	....
11/12/28.........	542	11.20	3.13	1.80	0.89	7.76	20.82	7.24	14.65	11.47	5.32
12/12/28	569	12.42	3.50	2.00	1.01	8.63	23.24	7.95	15.62	....	....
1/11/29.........	607	11.55	3.95	2.00	1.09	9.33	25.06	7.92	12.91	12.03	5.21
2/10/29.........	644	11.52	3.05	2.00	0.92	8.59	24.75	7.10	12.52	10.70	5.26
3/12/29.........	657	11.55	1.80	2.00	0.72	7.64	24.78	6.91	12.59	10.55	5.09
4/11/29.........	659	11.58	2.38	2.00	0.86	8.90	23.20	7.95	11.41	11.63	2.84
5/11/29.........	673	12.09	2.87	2.00	1.06	8.58	22.58	6.72	10.13	11.42	2.80
6/10/29.........	676	12.22	3.00	2.00	1.12	8.73	22.79	6.82	10.22	12.25	2.62
7/10/29.........	705	12.08	3.17	2.00	1.16	8.92	24.98	7.79	11.55	10.62	2.88
8/ 9/29.........	694	12.73	3.02	1.53	1.08	9.04	37.20	7.61	16.41	10.58	3.06
9/ 9/29.........	697	12.03	3.00	2.00	1.08	9.09	37.93	7.41	16.40	11.25	3.84
10/ 9/29.........	713	10.95	3.00	2.00	1.04	8.57	35.12	6.99	15.22	11.75	2.40
11/ 7/29.........	746	12.02	3.00	2.00	1.07	9.08	37.89	7.32	16.37	10.79	2.60

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days in the 30-day period.

Table 25

E 125, Group 1. Basal Ration, Low Phosphorus

Thirty-day period ending	Weight end of period	Daily ration			Nutrient intake per day		Mineral intake per day			Ca and P in 100 cc. blood plasma*	
		Prairie hay	Grain mixture	Molasses	Digestible proteins	Total digestible nutrients	Ca	P	Mg	Ca	Inorganic P
	lb.	lb.	lb.	lb.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
10/13/28.........	489	10.73	3.29	2.00	0.91	7.76	20.76	7.31	14.68	....	....
11/12/28.........	515	10.03	2.65	1.80	0.77	6.87	19.15	6.39	13.44	11.22	5.15
12/12/28.........	549	9.50	2.90	2.00	0.82	6.88	19.12	6.47	12.99	....	....
1/11/29.........	586	8.58	3.67	2.00	0.95	7.81	20.88	6.76	10.52	11.88	5.21
2/10/29.........	619	10.27	3.05	2.00	0.88	8.04	23.01	6.74	11.56	11.12	5.38
3/12/29.........	646	10.66	2.08	2.00	0.75	7.47	25.59	7.12	12.08	10.91	4.78
4/11/29.........	637	11.09	2.38	2.00	0.84	7.88	22.64	7.81	11.06	11.37	2.10
5/11/29.........	654	10.36	2.87	2.00	1.00	7.82	20.54	6.19	9.14	12.34	2.60
6/10/29.........	661	10.67	3.00	2.00	1.07	8.05	20.96	6.35	9.40	12.35	3.00
7/10/29.........	678	12.18	3.17	2.00	1.16	8.97	25.70	7.82	11.73	13.42	2.70
8/ 9/29.........	680	12.62	3.02	1.53	1.09	9.15	37.77	7.68	16.65	11.56	2.40
9/ 8/29.........	664	12.07	3.00	2.00	1.08	9.11	38.05	7.43	16.45	11.15	3.02
10/ 8/29.........	673	10.31	3.00	2.00	1.02	8.27	33.65	6.80	14.54	12.00	3.08
11/ 7/29.........	704	11.88	3.00	2.00	1.07	9.02	37.56	7.28	16.23	9.90	2.46

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days in the 30-day period.

Table 26

E 118, Group 2. Basal Ration, Low Phosphorus Plus 150 Grams Epsom Salt Daily

Thirty-day period ending	Weight end of period	Daily ration: Prairie hay	Daily ration: Grain mixture	Daily ration: Molasses	Daily ration: Epsom salt	Nutrient intake per day: Digestible proteins	Nutrient intake per day: Total digestible nutrients	Mineral intake per day: Ca	Mineral intake per day: P	Mineral intake per day: Mg	Ca and P in 100 cc. blood plasma*: Ca	Ca and P in 100 cc. blood plasma*: Inorganic P
	lb.	lb.	lb.	lb.	gm.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
10/13/28.........	876	15.58	4.21	1.08	...	1.19	10.38	24.94	9.44	17.52	....	....
11/12/28.........	875	13.83	3.48	1.86	...	1.02	9.20	24.65	8.43	17.26	10.81	5.44
12/12/28.........	926	12.90	3.50	2.00	73	1.02	8.84	23.88	8.12	23.38	....	....
1/11/29.........	971	13.20	3.95	2.00	150	1.14	10.06	27.36	8.40	28.98	11.83	4.62
2/10/29.........	996	14.39	3.05	2.00	150	1.00	9.86	28.76	7.95	29.54	10.40	5.23
3/12/29.........	1,042	15.92	2.08	2.00	145	0.90	9.79	30.93	8.67	30.48	9.95	4.65
4/11/29.........	1,036	16.25	2.30	2.00	150	0.99	10.08	29.12	9.22	29.11	10.35	3.59
5/11/29.........	1,054	14.77	2.86	2.00	135	1.14	9.75	25.75	7.53	24.97	11.17	3.46
6/10/29.........	1,044	14.18	3.00	2.00	150	1.19	9.59	25.11	7.42	26.13	12.50	3.22
7/10/29.........	1,079	15.24	3.26	2.00	150	1.26	10.31	29.05	8.75	28.28	9.46	3.54
8/ 9/29.........	1,084	15.42	2.64	1.53	131	1.08	10.19	44.68	8.13	32.46	9.76	1.85
9/ 9/29.........	1,083	16.79	3.00	2.00	150	1.21	11.35	49.79	8.88	36.34	12.15	3.06
10/ 8/29.........	1,110	13.82	3.00	2.00	150	1.12	9.94	42.38	7.88	33.12	11.40	2.89
11/ 7/29.........	1,130	14.08	3.00	2.00	150	1.13	10.06	43.03	7.96	33.41	10.05	2.88

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days in the 30-day period.

Table 27

E 121, Group 2. Basal Ration, Low Phosphorus Plus 150 Grams Epsom Salt Daily

Thirty-day period ending	Weight end of period	Daily ration				Nutrient intake per day		Mineral intake per day			Ca and P in 100 cc. blood plasma*	
		Prairie hay	Grain mixture	Molasses	Epsom salt	Digestible proteins	Total digestible nutrients	Ca	P	Mg	Ca	Inorganic P
	lb.	lb.	lb.	lb.	gm.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
10/13/28.........	631	13.29	3.58	2.00	...	1.03	9.09	24.10	8.41	16.24	....	....
11/12/28.........	677	13.50	3.50	1.80	....	1.02	9.05	24.01	8.32	16.83	10.96	5.01
12/12/28.........	698	13.40	3.50	2.00	73	1.04	9.21	25.04	8.36	25.15	....	....
1/11/29.........	734	11.85	3.95	2.00	150	1.10	9.47	25.48	8.01	27.93	10.66	5.54
2/10/29.........	784	13.09	3.05	2.00	150	0.96	9.28	26.94	7.57	28.53	10.55	6.45
3/12/29.........	804	14.60	1.80	1.80	145	0.80	8.87	28.20	7.76	29.00	10.10	5.22
4/11/29.........	799	14.34	2.38	2.00	150	0.94	9.31	26.70	8.78	27.92	10.61	3.47
5/11/29.........	816	14.09	2.87	2.00	135	1.12	9.45	24.94	7.32	24.58	10.96	3.31
6/10/29.........	811	11.33	3.00	2.00	150	1.10	8.34	21.74	6.55	24.51	10.00	3.75
7/10/29.........	848	11.85	3.17	2.00	150	1.15	8.82	24.64	7.72	26.91	11.21	3.70
8/ 9/29.........	823	11.26	3.02	1.53	150	1.05	8.50	34.38	7.26	29.98	9.86	3.60
9/ 8/29.........	832	10.10	3.00	2.00	150	1.02	8.17	33.13	6.82	29.11	11.25	3.51
10/ 8/29.........	841	11.24	3.00	2.00	150	1.05	8.71	35.95	7.08	30.93	11.05	3.14
11/ 7/29.........	866	12.13	3.00	2.00	150	1.07	9.14	38.18	7.36	31.30	10.84	2.59

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days in the 30-day period.

Table 28

E 122, Group 2. Basal Ration, Low Phosphorus Plus 150 Grams Epsom Salt Daily

Thirty-day period ending	Weight end of period	Daily ration				Nutrient intake per day		Mineral intake per day			Ca and P in 100 cc. blood plasma*	
		Prairie hay	Grain mixture	Molasses	Epsom salt	Digestible proteins	Total digestible nutrients	Ca	P	Mg	Ca	Inorganic P
	lb.	lb.	lb.	lb.	gm.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
10/13/28.........	688	13.38	3.58	2.00	...	1.03	9.12	24.42	8.43	17.14	....	....
11/12/28.........	739	13.45	3.32	1.80	...	0.98	8.89	23.92	8.12	16.73	10.02	5.20
12/12/28.........	775	13.32	3.50	2.00	73	1.03	9.02	24.46	8.24	23.67	....	....
1/11/29.........	800	11.38	3.97	2.00	150	1.09	9.28	24.83	7.88	27.59	11.62	5.84
2/10/29.........	874	13.65	3.05	2.00	150	0.98	9.53	27.72	7.73	28.96	10.30	6.62
3/12/29.........	887	14.28	2.08	2.00	145	0.85	9.06	28.63	8.18	29.18	10.00	6.40
4/11/29.........	886	15.03	2.38	2.00	150	0.97	9.61	27.56	8.99	28.35	10.66	4.04
5/11/29.........	912	13.76	2.87	2.00	135	1.11	9.31	24.56	7.23	24.39	10.96	3.82
6/10/29.........	939	13.80	3.00	2.00	150	1.18	9.43	24.66	7.31	25.92	12.00	4.08
7/10/29.........	972	16.20	3.17	2.00	150	1.29	10.75	30.91	9.06	29.11	10.90	4.09
8/ 9/29.........	971	16.57	3.02	1.53	150	1.20	11.02	47.59	8.90	35.71	10.82	3.66
9/ 8/29.........	967	15.72	3.00	2.00	150	1.18	10.84	47.13	8.55	35.19	10.50	3.85
10/ 8/29.........	976	11.55	3.00	2.00	150	1.06	8.86	36.73	7.18	30.67	12.75	3.40
11/ 7/29.........	1,027	12.17	3.00	2.00	150	1.08	9.15	38.26	7.37	31.34	11.18	3.33

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days in the 30-day period.

Table 29

E 124, Group 3. Basal Ration, Low Phosphorus Plus 150 Grams Epsom Salt and 65 Grams $NaH_2PO_4 \cdot H_2O$ Daily

Thirty-day period ending	Weight end of period	Daily ration					Nutrient intake per day		Mineral intake per day			Ca and P in 100 cc. blood plasma*	
		Prairie hay	Grain mixture	Molasses	Epsom salt	$NaH_2PO_4 \cdot H_2O$	Digestible proteins	Total digestible nutrients	Ca	P	Mg	Ca	Inorganic P
	lb.	lb.	lb.	lb.	gm.	gm.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
10/13/28......	791	14.92	4.04	2.00	...	..	1.15	10.16	26.59	9.37	18.68	....	....
11/12/28......	811	15.00	3.50	1.80	...	..	1.06	9.71	26.06	8.79	18.77	11.27	5.15
12/12/28......	848	14.73	3.50	2.00	73	29	1.07	9.64	26.42	15.15	24.87	....	....
1/11/29......	864	12.67	3.97	2.00	150	65	1.12	9.84	26.62	22.87	28.57	12.03	5.21
2/10/29......	955	13.80	3.05	2.00	150	65	0.98	9.60	27.93	22.58	29.08	10.15	7.72
3/12/29......	964	15.41	2.08	2.00	145	54	0.89	9.56	30.22	20.69	30.05	9.80	6.21
4/11/29......	981	15.73	2.38	2.00	150	65	0.99	9.92	28.42	23.81	28.78	10.20	4.77
5/11/29......	1,028	16.73	2.87	2.00	135	58	1.21	10.61	28.06	21.28	26.08	10.91	4.39
6/10/29......	1,063	13.43	3.00	2.00	150	65	1.16	9.27	24.22	21.80	25.71	11.25	5.18
7/10/29......	1,107	17.60	3.17	2.00	150	65	1.33	11.38	32.96	24.09	30.06	9.25	5.20
8/ 9/29......	1,124	17.93	3.02	1.53	150	65	1.24	11.67	51.00	23.93	37.19	10.31	5.37
9/ 8/29......	1,159	19.33	3.00	2.00	150	65	1.28	12.55	56.13	24.28	39.09	9.85	5.05
10/ 8/29......	1,172	15.27	3.00	2.00	150	61	1.16	10.63	46.00	22.00	34.69	10.90	4.95
11/ 7/29......	1,199	15.13	3.00	2.00	150	46	1.16	10.56	45.65	18.51	34.54	9.75	5.79

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days in the 30-day period.

Table 30

E 126, Group 3. Basal Ration, Low Phosphorus Plus 150 Grams Epsom Salt and 65 Grams $NaH_2PO_4 \cdot H_2O$ Daily

Thirty-day period ending	Weight end of period	Daily ration					Nutrient intake per day		Mineral intake per day			Ca and P in 100 cc. blood plasma*	
		Prairie hay	Grain mixture	Molasses	Epsom salt	$NaH_2PO_4 \cdot H_2O$	Digestible proteins	Total digestible nutrients	Ca	P	Mg	Ca	Inorganic P
	lb.	lb.	lb.	lb.	gm.	gm.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
10/13/28......	716	12.75	3.58	2.00	...	..	1.01	8.89	23.56	8.23	16.58	....	....
11/12/28......	737	11.97	3.10	1.80	...	..	0.90	8.07	21.86	7.44	15.32	11.27	4.53
12/12/28......	773	12.60	3.50	2.00	73	29	1.01	8.71	23.48	14.49	23.06	....	....
1/11/29......	808	11.28	3.83	2.00	150	65	1.06	9.13	24.67	22.33	27.46	11.88	6.00
2/10/29......	841	12.94	3.05	2.00	150	65	0.96	9.22	26.73	22.13	28.41	9.90	8.00
3/12/29......	885	14.03	2.08	2.00	145	54	0.85	8.96	28.29	20.29	28.99	9.38	6.67
4/11/29......	900	15.26	2.38	2.00	150	65	0.97	9.71	27.79	23.67	28.44	9.49	3.98
5/11/29......	948	16.46	2.87	2.00	135	58	1.20	10.49	27.74	21.20	25.93	10.61	4.83
6/10/29......	969	16.50	3.00	2.00	150	65	1.26	10.61	27.84	22.74	27.45	10.75	6.41
7/10/29......	1,022	17.16	3.17	2.00	150	65	1.32	11.18	32.22	23.95	29.72	11.01	5.40
8/ 9/29......	1,014	16.76	2.52	1.53	126	54	1.09	10.73	50.29	20.64	33.28	9.16	6.51
9/ 8/20......	1,034	17.21	3.00	2.00	150	65	1.22	11.55	50.85	23.63	36.80	10.31	6.92
10/ 8/29......	1,069	14.19	3.00	2.00	150	43	1.13	10.11	43.31	17.73	33.52	10.30	6.06
11/ 7/29......	1,107	13.85	3.00	2.00	150	46	1.12	9.95	42.45	18.11	33.15	9.20	6.75

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days in the 30-day period.

Table 31

E 127, Group 3. Basal Ration, Low Phosphorus Plus 150 Grams Epsom Salt and 65 Grams $NaH_2PO_4 \cdot H_2O$ Daily

Thirty-day period ending	Weight end of period	Daily ration					Nutrient intake per day		Mineral intake per day			Ca and P in 100 cc. blood plasma*	
		Prairie hay	Grain mixture	Molasses	Epsom salt	$NaH_2PO_4 \cdot H_2O$	Digestible proteins	Total digestible nutrients	Ca	P	Mg	Ca	Inorganic P
	lb.	lb.	lb.	lb.	gm.	gm.	lb.	lb.	gm.	gm.	gm.	mgm.	mgm.
10/13/28......	664	13.17	3.58	2.00	...	..	1.02	9.03	24.13	8.37	16.96	....	....
11/12/28......	723	12.18	3.03	1.80	...	..	0.90	8.11	22.15	7.44	15.49	10.92	4.91
12/12/28......	740	11.96	3.50	2.00	73	29	0.99	8.42	22.60	14.29	22.48	....	....
1/11/29......	789	10.98	3.97	2.00	150	65	1.07	9.10	24.27	22.38	27.27	12.03	6.95
2/10/29......	845	12.98	3.05	2.00	150	65	0.96	9.24	28.79	22.15	28.45	9.28	10.00
3/12/29......	890	15.16	2.08	2.00	145	54	0.98	9.45	29.86	20.62	29.85	9.38	5.94
4/11/29......	891	14.72	2.38	2.00	150	65	0.96	9.48	27.15	23.51	28.12	9.28	4.18
5/11/29......	962	16.25	2.87	2.00	135	58	1.19	10.40	27.50	21.13	25.81	10.56	5.10
6/10/29......	995	16.24	3.00	2.00	150	65	1.25	10.50	27.54	22.66	27.30	11.35	6.85
7/10/29......	1,013	16.60	3.17	2.00	150	65	1.30	10.93	31.28	23.78	29.30	11.41	5.40
8/ 9/29......	1,036	16.21	3.02	1.53	150	65	1.19	10.85	46.71	23.40	35.33	9.00	4.70
9/ 8/29......	1,061	17.35	3.00	2.00	150	65	1.23	11.62	51.20	23.67	36.95	10.15	6.49
10/ 8/29......	1,083	14.06	3.00	2.00	150	43	1.13	10.05	42.99	17.69	33.38	11.05	4.75
11/ 7/29......	1,118	13.85	3.00	2.00	150	46	1.12	9.95	42.45	18.11	33.15	8.91	6.75

* The Ca and P figures represent a composite sample made from blood samples drawn on three successive days during the 30-day period.

Table 32

Epsom Salt Experiment, Calcium and Phosphate Content of Blood Plasma

Averaged by groups.

	Calcium in 100 cc. blood plasma			Inorganic phosphorus in 100 cc. blood plasma		
	Group 1 check E120, E123, E125	Group 2 Epsom salt E118, E121, E122	Group 3 Epsom salt + $NaH_2PO_4 \cdot H_2O$ E124, E126, E127	Group 1 check E120, E123, E125	Group 2 Epsom salt E118, E121, E122	Group 3 Epsom salt + $NaH_2PO_4 \cdot H_2O$ E124, E126 E127
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
August	11.31	11.59	11.15	6.05	6.21	6.41
October	11.15	10.86	11.15	5.38	5.24	4.86
December	11.59	11.37	11.98	5.22	5.33	6.05
January	10.64	10.42	9.78	5.44	6.10	8.57
February	10.35	10.02	9.52	5.17	5.42	6.27
March	11.27	10.54	9.66	3.02	3.72	4.31
April	11.47	11.03	10.69	3.19	3.53	4.77
May	12.13	11.50	11.12	3.08	3.68	6.15
July	11.54	10.52	10.56	3.12	3.78	5.33
August	10.88	10.15	9.49	3.01	3.04	5.52
September	10.97	11.30	10.10	3.72	3.47	6.15
October	11.88	11.73	10.77	2.96	3.14	5.25
November	10.05	10.69	9.29	2.74	2.93	6.43

Table 33

E 120, May 8 to 17, 1929 (10 Days); Grade Holstein; Age Approximately 25 Months; Weight 889 Pounds; Blood Plasma, Total Ca 11.80, Inorganic P 3.61 mgm. in 100 cc.; Check Group, Low P, No Supplements

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie hay	54.48	0.460	0.094	0.208	250.61	51.21	113.32
Grain	13.62	0.091	0.196	0.116	12.40	26.70	15.80
Molasses	9.08	0.762	0.055	0.229	69.19	4.99	20.79
Water	177.06	*	....	....	8.68	....	.....
NaCl	0.91	....	....	....	.....	....	.....
Gross intake	.....	....	....	....	*340.88*	*82.90*	*149.91*
Refused feed	1.32	0.780	0.083	0.325	10.30	1.09	4.29
Net intake	.....	....	....	....	*330.58*	*81.81*	*145.62*
Excreta	204.67	0.149	0.033	0.077	304.96	66.52	157.60
Balance	.....	....	....	....	*+25.62*	*+15.29*	*−11.98*
Balance per day	.....	....	....	....	*+2.56*	*+1.53*	*−1.20*

* 49 parts Ca per 1,000,000.

Table 34

E 120, Sept. 12 to 21, 1929 (10 Days); Holstein; Approximate Age 29 Months; Weight 952 Pounds; Blood Plasma, Total Ca 10.50, Inorganic P 4.29 mgm per 100 cc.; Check Group, Low P, No Supplement

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie hay	54.43	0.522	0.087	0.225	284.13	47.35	122.47
Grain	13.61	0.082	0.225	0.083	11.16	30.62	11.30
Molasses	9.07	0.885	0.057	0.228	80.29	5.21	20.68
Water	212.05	*	....	....	10.39	....	.....
NaCl	1.13	....	....	....	.....	....	.....
Gross intake	.....	....	....	....	*385.97*	*83.18*	*154.45*
Refused feed	4.75	0.424	0.051	0.292	20.15	2.42	13.88
Net intake	.....	....	....	....	*365.82*	*80.76*	*140.57*
Excreta	263.34	0.136	0.030	0.074	358.14	78.47	194.87
Balance	.....	....	....	....	*+7.68*	*+2.29*	*−54.30*
Balance per day ..	.....	....	....	....	*+0.77*	*+0.23*	*−5.43*

* 49 parts Ca per 1,000,000.

Table 35

E 121, May 22 to 31, 1929 (10 Days); Grade Holstein; Age Approximately 25 Months; Weight 813 Pounds; Blood Plasma, Total Ca 10.0, Inorganic P 3.75 mgm in 100 cc.; low P Ration, Plus Epsom Salt

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie hay	54.58	0.432	0.100	0.303	235.35	54.48	165.07
Grain	13.62	0.123	0.225	0.110	16.75	30.65	14.98
Molasses..........	9.08	0.915	0.063	0.246	83.08	5.72	22.34
Epsom salt	1.50	....	....	10.350	.....	....	155.25
Water	228.36	*	....	....	11.19	....	.....
NaCl	0.91	....	....	....	.....	....	.....
Net intake	.....	....	....	....	*346.37*	*90.85*	*357.64*
Excreta	200.36	0.178	0.040	0.170	356.64	80.14	340.61
Balance	.....	....	....	....	*−10.27*	*+10.71*	*+17.03*
Balance per day ..	.....	....	....	....	*−1.03*	*+1.07*	*+1.70*

* 49 parts Ca per 1,000,000.

Table 36

E 121, Sept. 26 to Oct. 5. 1929 (10 Days); Holstein; Approximate Age 29 Months; Weight 839 Pounds; Blood Plasma, Total Ca 10.50, Inorganic P 4.29 mgm. per cc.; Low P Ration Plus Epsom Salt, NaCl *ad libitum*

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie hay	54.43	0.522	0.087	0.225	284.13	47.35	122.47
Grain	13.61	0.070	0.242	0.091	9.53	32.94	12.38
Molasses	9.07	0.885	0.057	0.228	80.29	5.21	20.68
Epsom salt	1.50	....	....	10.350	.....	....	155.25
Water	235.87	*	....	....	11.56	....	.....
NaCl	2.27	....	....	....	.....	....	....
Gross intake	.....	....	....	....	*385.51*	*85.50*	*310.78*
Refused feed	0.15	0.522	0.087	0.225	0.08	0.01	0.03
Net intake	.....	....	....	....	*385.43*	*85.49*	*310.75*
Excreta	225.56	0.182	0.040	0.144	410.52	90.22	324.81
Balance	.....	....	....	....	*—25.09*	*—4.73*	*—14.06*
Balance per day	.....	....	....	....	*—2.51*	*—0.47*	*—1.41*

* 49 parts Ca per 1,000,000.

Table 37

E 121, Nov. 23 to Dec. 2, 1929 (10 days); Holstein; Approximate Age 32 Months; Weight 871 Pounds; Blood Plasma; Total Ca 9.57, Inorganic P 4.00 mgm. per 100 cc.; Low P Ration, Epsom Salt Supplement, NaCl Limited to 1 oz. Daily

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie hay	54.43	0.525	0.082	0.235	285.76	44.63	127.91
Grain	13.61	0.041	0.229	0.084	5.58	31.17	11.43
Molasses	7.07	0.992	0.062	0.259	70.13	4.38	18.31
Epsom salt	1.50	....	....	10.350	.....	....	155.25
Water	182.34	*	....	....	8.93	....	.....
NaCl	0.28	....	....	....	.....	....	.....
Gross intake	.....	....	....	....	*370.40*	*80.18*	*312.90*
Refused feed	0.08	0.525	0.082	0.235	0.42	0.07	0.19
Net intake	.....	....	....	....	*369.98*	*80.11*	*312.71*
Excreta	172.30	0.224	0.048	0.185	385.95	82.70	318.76
Balance	.....	....	....	....	*—15.97*	*—2.69*	*—6.05*
Balance per day	.....	....	....	....	*—1.60*	*—0.27*	*—0.61*

* 49 parts Ca per 1,000,000.

Table 38

E 122, May 8 to 17, 1929 (10 Days); Grade Holstein, Approximate Age 25 Months; Weight 913 Pounds; Blood Plasma, Total Ca 12.00, Inorganic P 4.08 mgm in 100 cc.; Low P Ration Plus Epsom Salt

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie hay	54.48	0.460	0.094	0.208	250.61	51.21	113.32
Grain	13.62	0.091	0.096	0.116	12.40	26.70	15.80
Molasses	9.08	0.762	0.055	0.229	69.19	4.99	20.79
Epsom salt	1.50	....	....	10.350	.....	....	155.25
Water	190.68	*	....	....	9.53	....	.....
NaCl	0.91	....	....	....	.....	....	.....
Gross intake	.....	....	....	....	*341.73*	*82.90*	*305.16*
Refused feed	2.10	0.460	0.094	0.208	9.66	1.97	4.36
Net intake	.....	....	....	....	*332.07*	*80.93*	*300.80*
Excreta	222.02	0.153	0.035	0.143	339.69	77.71	317.49
Balance	.....	....	....	....	*—7.62*	*+3.22*	*—16.69*
Balance per day	.....	....	....	....	*—0.76*	*+0.32*	*—1.67*

* 49 parts Ca per 1,000,000.

Table 39

E 122, Sept. 12 to 21, 1929 (10 Days); Holstein; Approximate Age 29 Months; Weight 969 Pounds; Blood Plasma, Total Ca 10.50, Inorganic P 3.85 mgm per 100 cc.; Low P Ration Plus Epsom Salt

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie hay	54.43	0.522	0.087	0.225	284.13	47.35	122.47
Grain	13.61	0.082	0.225	0.083	11.16	30.62	11.30
Molasses	9.07	0.885	0.057	0.228	80.29	5.21	20.68
Epsom salt	1.50	....	....	10.350	.....	....	155.25
Water	176.90	*	....	....	8.67	....	.....
NaCl	1.13	....	....	....	.....	....	.....
Gross intake	.....	....	....	....	*384.25*	*83.18*	*309.70*
Refused feed	2.50	0.559	0.064	0.276	13.98	1.60	6.90
Net intake	.....	....	....	....	*370.27*	*81.58*	*302.80*
Excreta	242.91	0.151	0.030	0.136	366.68	72.87	330.36
Balance	.....	....	....	....	*+3.59*	*+8.71*	*—27.56*
Balance per day	.....	....	....	....	*+0.36*	*+0.87*	*—2.76*

* 49 parts Ca per 1,000,000.

Table 40

E 124, May 21 to 31, 1929 (10 Days); Grade Holstein; Approximate Age 25 Months; Weight 1,044 Pounds; Blood Plasma, Total Ca 11.25, Inorganic P 5.18 mgm in 100 cc.; Low P Ration Plus Epsom Salt and $NaH_2PO_4 \cdot H_2O$

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie hay	54.48	0.432	0.100	0.303	235.35	54.48	165.07
Grain	13.62	0.123	0.225	0.110	16.75	30.65	14.98
Molasses	9.08	0.915	0.063	0.246	83.08	5.72	22.34
Epsom salt	1.50	....	....	10.350	.....	....	155.25
$NaH_2PO_4 \cdot H_2O$	0.65	22.481	....	....	.....	146.22	.....
Water	236.08	*	....	....	11.57	....	.....
NaCl	0.91	....	....	....	.....	....	.....
Net intake	.....	....	....	....	*346.75*	*237.07*	*357.64*
Excreta	225.47	0.139	0.088	0.146	313.40	198.41	329.19
Balance	.....	....	....	....	*+23.35*	*+36.66*	*+28.45*
Balance per day	.....	....	....	....	*+2.34*	*+3.67*	*+2.85*

* 49 parts Ca per 1,000,000.

Table 41

E 124, Sept. 26 to Oct. 5, 1929 (10 Days); Holstein; Approximate Age 29 Months; Weight 1,169 Pounds; Blood Plasma, Total Ca 9.95, Inorganic P 5.05 mgm per 100 cc.; Low P Ration Plus Epsom Salt and $NaH_2PO_4 \cdot H_2O$, NaCl *ad libitum*

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie hay	68.04	0.522	0.087	0.225	355.17	59.19	153.09
Grain	13.61	0.070	0.242	0.091	9.53	32.94	12.38
Molasses	9.07	0.885	0.057	0.228	80.29	5.21	20.68
Epsom salt	1.50	....	....	10.350	.....	....	155.25
$NaH_2PO_4 \cdot H_2O$	0.65	....	22.481	....	.....	146.22	.....
Water	235.87	*	....	....	11.56	....	.....
NaCl	2.72	....	....	....	.....	....	.....
Gross intake	.....	....	....	....	*456.55*	*243.56*	*341.40*
Refused feed	1.25	0.645	0.050	0.312	8.06	0.63	3.90
Net intake	.....	....	....	....	447.49	242.93	337.50
Excreta	260.86	0.160	0.079	0.152	417.38	206.08	396.51
Balance	.....	....	....	....	*+30.11*	*+36.85*	*—59.01*
Balance per day	.....	....	....	....	*+3.01*	*+3.69*	*—5.90*

* 49 parts Ca per 1,000,000.

Table 42

E 124, Nov. 23 to Dec. 2, 1929 (10 Days); Holstein; Approximate Age 31 Months; Weight 1,225 Pounds; Blood Plasma, Ca 10.20, Inorganic P 4.67 mgm per 100 cc.; Low P Ration, Epsom Salt, $NaH_2PO_4 \cdot H_2O$ Supplements, NaCl Limited to 1 oz. Daily

	Weight	Analysis			Mineral content		
		Ca	P	Mg	Ca	P	Mg
	kg.	per cent	per cent	per cent	gm.	gm.	gm.
Prairie Hay	68.04	0.525	0.082	0.235	357.21	55.79	159.89
Grain	13.61	0.041	0.229	0.084	5.58	31.17	11.43
Molasses	9.07	0.992	0.062	0.259	89.97	5.62	23.49
Epsom salt	1.50	....	....	10.350	.....	....	155.25
$NaH_2PO_4 \cdot H_2O$ ...	0.65	....	22.481	....	.....	146.22	.....
Water	215.91	*	....	....	10.58	....	.....
NaCl	0.27	....	....	....	.....	....	.....
Gross intake	.....	....	....	....	*463.34*	*238.80*	*350.06*
Refused feed	0.30	0.525	0.082	0.235	1.58	0.25	0.71
Net intake	.....	....	....	....	*461.76*	*238.55*	*349.35*
Excreta (dry)	42.40	1.063	0.498	0.913	450.71	211.15	387.11
Balance	.....	....	....	....	*+11.05*	*+27.40*	*—37.76*
Balance per day ..	.....	.....	....	....	*+1.11*	*+2.74*	*—3.78*

* 49 parts Ca per 1,000,000.

Table 43

Chemical Analyses of Feeds Used in Magnesium Sulfate Experiments

Feed	Lot No.	Date fed from	Date fed to	Ash	Crude fiber	Protein	Ether extract	N-free extract	Ca	P	Mg
				per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Prairie hay	25	9/20/28	11/30/28	7.14	25.67	5.26	3.37	43.77	0.301	0.069	....
" "	26	12/1/28	3/22/29	6.63	25.99	5.80	3.42	42.99	0.307	0.065	....
" "	27	3/23/29	7/5/29	7.69	24.19	6.41	3.24	44.42	0.260	0.067	....
" "	29	7/6/29	11/27/29	4.59	28.39	5.62	4.23	45.60	0.549	0.068	....
" "	28	11/18/29	8/4/30	7.13	24.98	5.43	3.87	43.32	0.603	0.070	0.238
Corn gluten meal	1	9/23/28	2/10/29	1.08	1.44	38.48	1.88	46.99	0.015	0.115	0.232
" " "	6	2/11/29	4/11/29	3.46	5.26	43.14	2.91	38.00	0.029	0.523	0.025
" " "	9*	4/1/29	11/20/29	1.36	4.98	41.79	2.47	37.72	0.058	0.142*	0.166
Corn	8	9/20/28	10/13/29	1.12	1.51	9.03	3.93	69.03	0.015	0.222	0.050
"	9	10/14/28	1/11/29	1.27	2.16	9.31	3.05	69.39	0.015	0.301†	....
"	10	1/12/29	2/10/29	1.18	2.34	8.79	3.37	65.92	0.005	0.301†	....
"	11	2/11/29	6/10/29	1.23	2.21	8.41	3.76	65.46	0.012	0.232	....
"	12	6/11/29	8/19/29	1.15	2.67	9.46	4.21	69.17	0.009	0.241	0.100
"	15	8/20/29	11/20/29	1.12	2.63	9.25	3.41	70.46	0.008	0.217	0.116
Oats	14	9/20/28	9/3/28	3.82	9.86	12.15	2.46	63.10	0.292	0.063	0.131
"	15	9/4/28	1/11/29	3.02	9.47	12.58	4.32	56.19	0.129	0.218	0.138
"	16	1/12/29	6/10/29	3.32	9.48	12.50	4.20	59.23	0.091	0.267	0.127
"	13‡	6/11/29	8/19/29	3.50	10.90	12.40	4.40	59.60	0.100	0.353	0.100
"	17	8/20/29	11/20/29	3.40	10.52	12.38	3.73	60.38	0.075	0.261	0.114

* This lot washed to reduce the phosphorus content.
† Phosphorus determination not made, average figures used.
‡ Not analyzed, average analyses used.